Study of the incidence of muco-cutaneous Graft versus Host Disease among patients undergoing allogeneic hematopoietic stem cell transplantation in an Indian setting

Submitted by
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CERTIFICATE

This is to certify that the dissertation entitled 'Study of the incidence of muco-cutaneous Graft versus Host Disease among patients undergoing allogeneic hematopoietic stem cell transplantation in an Indian setting' is the bonafide original work of **Dr. Anisha Chandy.**

This study was undertaken at the **Christian Medical College, Vellore** from the year 2009 under my direct guidance and supervision, in partial fulfillment of the requirement for the award of the MD degree in Dermatology, Venereology and Leprosy of the Tamil Nadu Dr. M.G.R Medical University.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) has evolved from an experimental option to standard therapy in many congenital and acquired hematopoietic disorders as well as malignancies. More than 30,000 allogeneic HSCTs are done every year, with a likely increase by 5-10% each year.[1] Rates of transplantation continue to increase for all indications.[2] Graft versus Host Disease (GVHD) is a major complication of HSCT. It is defined as the aggregation of clinical and pathological manifestations in a recipient of allogeneic stem cells or bone marrow transplantation in which specific immunological as well as nonspecific phenomena lead to characteristic features. Based on the time of presentation, it has been traditionally classified as acute and chronic GVHD.[3] Acute GVHD appears within 100 days of the transplant and chronic GVHD after 100 days.[4] The usual presentation of acute GVHD is a maculopapular rash.[5] The spectrum of chronic GVHD includes lichenoid and sclerodermoid forms, the latter containing lichen sclerosus, morphea, panniculitis and fasciitis.[6]

Incidence of GVHD can be upto 80%, depending on human leukocyte antigen (HLA) mismatch, age, gender and conditioning regimen.[4] Despite success in decreasing the incidence of acute GVHD (eg. via prophylaxis with immunosuppressive medications), the incidence of chronic GVHD has increased from approximately 40% in 1990 to 80% today.[7,8]

Risk factors for GVHD include:

1. Characteristics of the donor and recipient: HLA disparity, unrelated HLA-matched donor (mismatched minor histocompatibility antigens), female donor to male recipient, older age of donor or recipient and prior acute GVHD

2. Characteristics of the transplantation protocol: more intense preparative regimen (myeloablative vs. reduced-intensity conditioning regimens), source and dose of hematopoietic stem cells- G-CSF-mobilized peripheral blood rich in CD34+ cells, unmodified (T-cell-replete) graft; less aggressive administration of prophylactic immunosuppressive agents (to prevent GVHD)
3. Later interventions to incite a graft-versus-malignancy effect, namely withdrawal of immunosuppressive drugs and donor T lymphocyte infusions.[9]

The incidence of GVHD in Western literature varies widely. Studies in Japan and Taiwan have shown a lower incidence, attributed to lesser genetic diversity. We undertook a study to determine the incidence of graft versus host disease, describe the various clinical presentations of muco-cutaneous GVHD, establish the clinico-pathological correlation of acute cutaneous GVHD and analyze the risk factors for GVHD in a tertiary referral center in India.

AIMS AND OBJECTIVES

1. To estimate the incidence of muco-cutaneous GVHD in a tertiary referral center in south India.
2. To describe the various clinical presentations of muco-cutaneous GVHD.
3. To establish the clinico-pathological correlation of acute cutaneous GVHD.
4. To analyze the risk factors for GVHD.

REVIEW OF LITERATURE

Introduction:

GVHD is a major complication of HSCT. GVHD was described very early in the history of HSCT and is a donor-derived immunological response against recipient tissue antigens.[1] HSCT has been integrated into treatment algorithms as first line therapy in aplastic anemia, after 1st complete remission in high risk acute leukemias, and as rescue therapy in non-responders, relapsed or aggressive leukemias and lymphomas.[1]

Types of HSCT:

The types of transplants (based on donor type) can be autologous, when patient's own cells are used for transplant; syngeneic, when the cells of a twin are used; allogeneic, when cells from another person are used as stem cell source, either from the same family or from an unrelated donor.

Depending on whether the HLA, that is, the major histocompatibility complex (MHC), is matched or not, the donor can be an HLA identical sibling, or other family member, a non-HLA identical family member, or an unrelated donor, either HLA matched or mismatched.[3] The perfect HLA match or the 10/10 allele match is a sibling donor with identical alleles of the HLA-A, -B, -C, DRB1 and DQB1 loci. The total number of mismatched alleles, locus of the mismatched allele and resolution of the mismatch determine the effect of outcome.[10]

HSCT is of different types based on the site and mode of harvest; bone marrow transplantation, in which stem cells are collected from bone marrow by repetitive puncture of the bone marrow; peripheral blood stem cell transplantation, in which stem cells are collected from peripheral blood with a cell separator after mobilization of stem

cells with G-CSF to the peripheral blood; and cord blood transplantation, in which stem cells are collected from umbilical cord blood and placenta of the newborn immediately after delivery.[3] Peripheral blood stem cell transplants (PBSCT) are being performed increasingly due to the ease of collection, faster engraftment kinetics, less immediate morbidity associated with stem-cell procurement, similar clinical outcome and economical advantages.[11] The advantages of using cord blood as stem cell source are absence of risk to the donor, immediate availability of cells, low risk of transmitting infections and a low risk of GVHD.[12]

Sequence of events:

The sequence of events in HSCT includes pre-transplant conditioning, and the transplant procedure itself.[1] The conditioning regimen is the pre-transplantation treatment with chemo-radiotherapy to reduce or eradicate the tumor burden and immunosuppress the host in order to allow engraftment of the transplant, and creates space for the graft.[1,3] It can be either myeloablative conditioning (traditional regimen), in which primary targets are the elimination of the tumor cells as well as the induction of a state of immunosuppression in the host, or a reduced intensity conditioning, in which the primary target is to induce a state of immunosuppression in the host; elimination of the recipient stem cells as well as of residual tumor cells is mainly performed by immunocompetent donor cells.[3] Nonmyeloablative conditioning reduces the damage to the host's tissues and can maintain a transient or prolonged state of hematopoietic-cell chimerism, which is an indication of immunologic tolerance.[13] Hematopoietic chimerism refers to all marrow cells of the recipient being of donor origin, whereas all the other tissues are native to the host.[14]

The transplant procedure consists of infusing the stem cells harvested from bone marrow, peripheral blood or cord blood intravenously.[1] After infusion of stem cells, they home to the marrow and reconstitute the immunologic and hematopoietic system of the recipient.[14]

Engraftment:

As the graft begins to function, during neutrophil recovery, a wave of cytokine function without concomitant T cell mediated attack, results in fever, rash and fluid retention, in the form of pulmonary edema, referred to as "engraftment syndrome" or "capillary leak syndrome". It occurs in the 1st 1 to 2 weeks of the transplant and may be associated with increased mortality, especially from pulmonary failure. Prompt improvement with corticosteroids is characteristic.[15]

Graft versus host disease

GVHD is a clinical syndrome, initially described as "runt disease" in mice.[16] The first published report was probably in 1960, when transplants were used to treat nuclear accident survivors.[15] GVHD is caused by the recognition of major and/or minor histocompatibility antigen differences by alloreactive T-lymphocytes in the stem cell graft.[17] Cutaneous GVHD is one of the most common complications of allogeneic HSCT.[18] Clinically significant disease occurs in about 50% patients,[14,19] ranging between 35-75%.[20] It can occur in about 8% patients undergoing autologous transplantation.

Etiology:

The original pre-requisites of GVHD as described by Billingham, in 1966, stated that the transplanted graft must contain immunologically competent cells, the recipient must express tissue antigens that are not present in the transplant donor and the recipient

must be incapable of rejecting the transplanted cells.[21] These tenets were revised in 2006, to include the effector cells migrating to the target tissues.[22]

Histocompatibility:

Class II HLA molecules (DR, DQ and DP) are presented by $CD4^+$ T cells, thus helping them to recognize foreign antigens. In antigen presenting cells (APCs), the dimerization of DR1 molecule may induce co-stimulatory molecule expression.[5] Minor histocompatibility antigens (miH) are peptides recognized by donor T cells that are derived from intracellular proteins and presented by MHC molecules.[23] After the presentation of miH by the MHC to the donor T cells, the non-self peptide bound to the MHC molecules trigger the $CD8^+$ T cell in case of MHC class I and $CD4^+$ T cell in case of MHC class II thereby inducing GVHD.[5] The minor histocompatibility antigens HY and HA-3 are targets for GVHD.[24]

Pathophysiology:

The current concept explaining the mechanism of GVHD is that phenomena namely alloreactivity, autoimmunity and immunodeficiency occur in differing intensities, alloreactivity mainly involved in the acute stage and autoimmunity in the chronic stage, depicted in **Figure 1**.[3]

Acute GVHD:

Two principles govern the pathophysiology of acute GVHD, typical inflammatory mechanisms are exaggerated in the foreign environment of host they are infused into and recipient's tissues that are damaged by underlying disease, conditioning regimen and prior infections stimulate the donor cells.[24] There are three sequential stages for the development of GVHD, depicted in **Figure 2**.[3] The first two stages form the afferent

phase and the last stage, the effector phase. The afferent phase starts even before the infusion of the graft and is at the time of conditioning where the patient receives total body irradiation (TBI) or high dose chemotherapy as part of a myeloablative regimen and to suppress the host's ability to reject the graft. Damaged tissues express inflammatory cytokines like IL-1, -6, TNF-α, GM-CSF and IFN-γ which upregulate adhesion molecules and enhance MHC antigens.[5] Intestinal mucosa is mainly damaged by the conditioning, leading to translocation of lipopolysaccharides (LPS) from the lumen into the circulation, which causes cytokine expression.[25]

The second phase involves donor T cells recognizing foreign host antigens, getting activated and stimulated. Host APCs stimulate T cells via cytokines like IL-1 and IL-2 and are essential for this phase of GVHD. Adhesion molecules involved in T cell adhesion and activation include E-selectin, VLA-4, LFA-1, ICAM-1, PECAM-1 and VECAM-1.[5] There are genetically distinct epithelial cells in the basal layer which are confined to the rete ridges in the skin and rete-like prominences in the dorsal tongue known as selectively targeted apoptotic rete (STAR) cells, which preferentially express adhesion molecules like CD106 and a specific cytoskeletal protein termed cytokeratin 15.[26]

In the effector phase of GVHD, there is direct and indirect damage to the host cells. A predominance of T helper type 1 (Th1) cells leads to activation of cytotoxic T lymphocytes (CTLs), and the presence of a cytokine storm, including IL-1, -2, -8, TNF-α and IFN-γ. CTLs and natural killer (NK) cells mediate cytotoxicity via TNF-α, perforin-granzyme B and Fas-Fas ligand.[5] LPS and other stimulatory molecules from the intestinal mucosa trigger immune cells through secondary signals. Complex interactions between dendritic cells and immune effectors play a major role in the development of GVHD.[25]

Chronic GVHD:

Macrophages mainly produce TGFβ in the skin, which is critical in Sclerodermatous (Scl) GVHD.[27] In early fibrosing disease there is a Th1 environment with cytokines like IFNγ, IL-2 and -18,[27] chemokines like CXL9/Mig, CXCL10/IP-10 and CXCL11/I-TAC and growth factors like PDGF, FGF1, EGF and nerve growth factor-β.[28] Later, a T helper type 2 (Th2) profile predominates with CCL17, CTGF and VEGF.[28] Hypotheses on pathogenesis include thymic damage by acute GVHD leading to failure to delete nascent T cells which recognize antigens, TGFβ, B cell and antibody mediated mechanisms and dysfunctional T-regulatory cells.[29]

<u>Risk factors</u>:

Acute GVHD:

The risk factors of acute GVHD include HLA and miH antigen mismatch, high dose chemotherapy or TBI as conditioning, presence of intestinal anaerobic bacterial microflora, advanced age, gender mismatch, especially female multiparous donor with male recipient, multiple transfusions, underlying primary disease especially chronic myeloid leukemia (CML), positive cytomegalovirus serology, splenectomised recipient, dose of immunosuppressive prophylactic regimen[5] and number of T cells in the graft.[30] Peripheral blood rather than bone marrow transplant is a risk factor for chronic, not acute GVHD.[5] The main risk factor is histoincompatibility. H-Y antigens are expressed by male recipients on their Y chromosome, and are recognized as foreign by female donor cells.[15] In a HLA matched sibling donor transplant, the risk of grade II to IV GVHD was upto 50%, but increased to 60% with disparity of one antigen, 75% with two and 90% with three antigens.[14] A multivariate analysis revealed that incompatibility with respect to HLA-A and HLA-C alleles between the donor and host were independent strong risk

factors for the development of severe acute GVHD.[31] A single mismatch between either HLA-A, -B, -C or -DR locus correlated with increased incidence of severe acute GVHD; whereas HLA-A and/or -B resulted in increased chronic GVHD.[32]

A high degree of donor chimerism is associated by the development of GVHD, probably due to reduced-intensity conditioning regimens. Other controversial risk factors include exposure to herpes viruses, $CD34^+$ cell dose, certain HLA alleles and ABO incompatibility.[15] Granulocyte colony-stimulating factor (G-CSF) was associated with increased risk of grade II acute GVHD in HLA matched sibling transplants despite prophylaxis with cyclosporine and methotrexate.[33] There is conflicting data on the use of stimulating factors like G-CSF and GM-CSF and the incidence of GVHD.[34]

Studies done to assess risk factors in acute GVHD are summarized in **Table 1**.

Table 1. Studies assessing risk factors of acute GVHD:

Author [Ref.]	**Risk factor**
Vargas-Diez E et al[35]	CML TBI HLA disparity
Nash RA et al[36]	Gender mismatch, especially with a parous donor Myeloablative conditioning Disease status
Hahn T et al[37]	CML Older age

Chronic GVHD:

The most important risk factors for chronic GVHD are prior acute GVHD, prophylactic regimen for acute GVHD, age of the recipient being more than 20 years, T

cell replete graft, second marrow infusion, transfusion of non-irradiated donor buffy coat, female donor (especially parous) for male host and a history of herpes infection.[38,8] Controversial risk factors include patient characteristics like CMV seropositivity or reactivation and splenectomy, donor characteristics like ethnic difference between donor and host and the presence of corticosteroids or lack of Methotrexate in the acute GVHD prophylaxis regimen and high $CD34^+$ count (PBSCT).[8] A positive random skin biopsy, in the absence of symptoms or a history of previous acute GVHD predicts the risk of chronic GVHD to be three times.[34]

The risk of chronic GVHD has increased as more patients with increasing age and co-morbidities are taken for HSCT, the eligibility criteria for the same has been expanded, there is increased HLA disparity, prolonged survival due to better prophylactic drugs for acute GVHD and treatment of other early complications, and increased use of peripheral blood stem cells rather than bone marrow for infusion.[3,7] T cell depletion, cord blood transplantation and Antithymocyte globulin (ATG) decrease the incidence of acute GVHD, but don't prevent chronic GVHD.[7,9]

Incidence:

Incidence of GVHD can be upto 80%, depending on HLA mismatch, age, gender and treatment regimen,[4] occurs in two-third of HLA identical sibling transplants and one-fifth of them are severe.[1] The incidence of acute GVHD in thalassemia was 26.9% in a single center in Italy.[39] The incidence of GVHD in children is less than that of adults.[40] The reported incidences of acute and chronic GVHD are depicted in **Tables 2** and **3** respectively.

Table 2. Reported incidence of acute GVHD:

Acute GVHD			
Author [Ref.]	**Year**	**Incidence**	
Mauduit G et al[41]	1988	50-80%	
Aractingi S et al[42] Fimiani M et al[38]	1998 2003	Mean 35% 6-90%	
Goker H et al[5]	2001	HLA matched 50-90%	
Ferrara JLM at al[24]	2009	HLA matched 40%	
Ringden O et al[43]	1995	HLA matched sibling 11%	Matched unrelated donor 15%
Tabbara IA et al[44]	2002	HLA Matched 20-50%	HLA mismatched sibling or matched unrelated donor 50-80%

Table 3. Reported incidence of chronic GVHD :

Chronic GVHD				
Author (Reference)	**Year**	**Incidence**		
Matsuoka LY et al[45]	1981	Upto 25%		
Mauduit G et al[41]	1988	30%		
Penas PF et al[46]	2010	60-80%		
Fujii H et al[47]	1992	32%	No difference if prior acute GVHD	
Fimiani M et al[38]	2003	30-50%	With prior acute GVHD 70%	
Sullivan KM et al[48]	1991	HLA matched 33%	Mismatched 49%	Matched unrelated donor 64%
Ringden O et al[43]	1995	HLA matched sibling 22%	Matched unrelated donor 29%	
Ilhan O et al[49]	1997	HLA matched 30-55%	Mismatched 70-90%	
Horwitz ME et al[34]	2006	1 antigen HLA-non-identical unrelated donor Upto 80%		

In the study done in Japan, the differences compared to Western data were attributed to histocompatibility antigens having a lesser degree of genetic diversity.[47] A study in Taiwan had a reduced prevalence of grade II to IV acute GVHD, again attributed to lower levels of genetic diversity and to isolation rooms which had laminar airflow.[50]

Clinical features:

The diagnosis of GVHD is clinical; no reliable laboratory tests can confirm or refute the diagnosis.[15]

Hyperacute GVHD:

Hyperacute GVHD, a fulminant, rare form of disease, is seen in the 1st week after transplant with fever, erythroderma, hepatitis and vascular leakage.[5] It is also known as early mismatch GVHD.[3] It was found in 27% of grade II to IV acute GVHD; can occur even before neutrophil engraftment and is associated with a reduced response to standard therapy, with increased non-relapse mortality, in matched unrelated donor or mismatched related donor transplants.[51]

Acute GVHD:

Acute GVHD usually occurs between two and six weeks after transplant,[5] most commonly between day 7 and 21,[38,42] median onset being day 19.[52] With high intensity conditioning, it occurs between 14 and 35 days, with GVHD prophylaxis of Cyclosporine and Methotrexate, median onset is 21 to 25 days after transplant.[15] The peak incidence is around day 30 after myeloablative conditioning.[3]

Acute GVHD is rather organ specific, the lymphocytes enter the target tissue as they possess the required combination of homing and chemokine receptors to engage the

endothelium.[22] The target organs of acute GVHD are skin, liver, gut, lung and lymphoid tissue.[46] The skin, gut and liver are affected in decreasing order of frequency.[40] Extracutaneous acute GVHD is almost always accompanied by skin disease.[14]

Skin:

The expression of acute GVHD in the skin is called acute cutaneous graft versus host reaction.[53] The skin can be the sole target of acute GVHD.[3] The most common and earliest clinical presentation of acute GVHD is a pruritic maculopapular rash involving the ears, nape of neck, shoulders, palms and soles in the early stage, resembling a sunburn,[5] spreading to the cheeks and upper trunk,[30] which can progress to erythroderma and even epidermal necrolysis.[5] Bullae can appear with a positive Nikolskiy sign.[3] The symmetrical morbilliform rash usually begins acrally and becomes disseminated. The characteristic features include acral erythema, violaceous discoloration of the ears and folliculocentric erythema that blanches with small macules and papules.[3] The rash can be asymptomatic or painful.[15] A rash usually precedes gastrointestinal and liver involvement,[54] and is associated with fever and pancytopenia.[38] Acute GVHD can also present as a scarlatiniform rash or a rash similar to varicella infection,[42] ichthyosiform eruptions[46] or acute psoriasiform cutaneous GVHD.[55] It can rarely present with discrete, erythematous to violaceous follicular papules[56] with central hyperkeratosis.[57] Oral, conjunctival and vaginal lesions which don't heal with hematological recovery may represent mucosal GVHD.[15] Manifestations attributed to oral acute GVHD include punctate or generalized mucosal erythema, mucosal erosions, desquamation, ulceration, mucoceles, xerostomia and white striae similar to lichen planus. Most commonly, the tongue, labial and buccal mucosa are involved.[52]

A retrospective study done on 15 patients with stage IV acute GVHD revealed that the patterns of evolution could be a slow progression from an erythematous rash to vesiculobullous lesions, where numerous vesicles were seen or flaccid bullae on frictional areas were noted or quick progression from an erythematous rash to extensive denudation of skin. Median interval between onset of a rash and appearance of vesicles was 19 days. Mucosal involvement in the form of oral erosions was most common.[18]

Differential diagnosis:

Patients who have more than 60% body surface area involvement with an erythematous rash have a high prevalence of GVHD.[58] The differential diagnosis for acute cutaneous GVHD includes drug rash, viral exanthem and the eruption of lymphocyte recovery. There can be a considerable overlap between clinical and histological features of these conditions.[9] Eruption of lymphocyte recovery (ELR) also presents as macules and papules between day 14 and 21. This time corresponds to the earliest re-appearance of lymphocytes in the peripheral circulation after marrow ablation. Impending immunologic reconstitution can be predicted by identifying this eruption.[53] Cause of this eruption may be due to unopposed reactivity to autoantigens or due to homing of returning lymphocytes in the skin. On an average, ELR is seen earlier than GVHD.[59] Involvement of palms and soles in characteristic of GVHD, but can also occur due to the conditioning regimen, more commonly with Busulphan, manifesting as painful, blistering acral erythema, resembling a second degree burn. Severe acute GVHD doesn't usually involve the conjunctiva, hence helping to differentiate it from toxic epidermal necrolysis.[15]

Liver:

The liver is the second most common organ to be involved in acute GVHD, manifesting with conjugated hyperbilirubinemia and elevation of alkaline phosphatase in the early stages.[5] Transaminase abnormalities are less common.[15]

Gut:

The gastrointestinal (GI) tract is the third most commonly involved organ in GVHD and the most severe and difficult to treat. Any site can be involved, presentation is with diarrhea, abdominal cramps, nausea, vomiting, distension, ileus, bleeding,[5] anorexia, malabsorption and ascites.[42] The colon is more commonly involved than the ileum.[15] Acute upper GI GVHD in older patients presents with anorexia, nausea, vomiting, dyspepsia and food intolerance, and is more responsive to treatment.[5]

Other sites:

Presence of acute lung GVHD is still controversial.[15] GVHD in lymph nodes leads to diminution of germinal centers. GVHD may cause thrombocytopenia.[5]

Grading:

An overall GVHD grade is the composite score of the skin, liver and gut stage. The histological grade is different from the clinical grade. Staging of acute GVHD adapted from consensus conference, depending on the extent of organ involvement is represented in **Table 4**.[60-62]

Table 4. Staging of acute GVHD adapted from the consensus conference**:**

Stage	Skin	Liver	Gut
1	Rash<25% of skin (body surface area according to the rule of nines)	Bilirubin 2-3mg/dl	Diarrhea>500ml/day or persistent nausea with biopsy confirming GVHD
2	Rash 25-50%	Bilirubin 3-4mg/dl	Diarrhea >1000ml/day
3	Rash >50% of skin	Bilirubin 6-15mg/dl	Diarrhea>1500ml/day
4	Generalized erythroderma with bullae	Bilirubin>15mg/dl	Severe abdominal pain ± ileus

Criteria for grading are given as minimum degree of organ involvement for each grade, shown in **Table 5**.

Table 5. Grading of acute GVHD adapted from the consensus conference**:**

Grade	Skin	Liver	Gut
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2-3 or	Stage 2-4
IV	Stage 4 or	Stage 4	-

The consensus grading and International Bone Marrow Transplant Registry (IBMTR) GVHD severity index are in use now.[15] Revised criteria were proposed for grading acute GVHD to categorize clinical management, as there was considerable disagreement in assigning acute GVHD grades by the original criteria by independent reviewers. This new criteria has limited prognostic utility and is shown in **Table 6**.[63]

Table 6. Revised criteria for grading acute GVHD:

Grade	Features
I	No convincing evidence of GVHD at any time; all abnormalities in skin, liver and gut fully accountable by processes other than GVHD; no immunoprophylaxis given except for originally planned prophylaxis.
II	Rash characteristic of GVHD in clinical presentation and time of onset (with or without visceral GVHD) or biopsy proven visceral GVHD without rash; improvement without need for treatment or progressive improvement within 2-3 weeks after starting treatment; no need for secondary treatment.
III	Clinical presentation as described for grade II but without progressive improvement after starting treatment, or requiring multiple cycles of treatment or extended hospitalization, but without GVHD as a clinically significant contributing cause of death.
IV	Clinical presentation as described in grade II but with GVHD as a clinically significant contributing cause of death.

Traditionally GVHD occurring after 100 days was considered chronic, but now chronic GVHD is characterized by clinical features rather than temporal onset.[64] Features of acute GVHD can occur after day 100 and clinical features of chronic GVHD even shortly after transplant,[3] and has been recorded as early as 40 days after transplant.[42] Hence, these criteria should be used only as a guide.[65] Subcategories include classic acute, persistent, recurrent or late onset acute, classic chronic and overlap syndrome. Persistent, recurrent or late onset acute GVHD usually occurs on withdrawal of immunosuppression.[9,66] Patients with late acute GVHD had gut and liver involvement more often and skin involvement less often compared to patients with acute GVHD.[67] Patients with overlap syndrome have more extensive disease.[68]

Categories of acute and chronic GVHD, from the National Institute of Health (NIH) consensus are depicted in **Table 7**.[66]

Table 7. Categories of acute and chronic GVHD, NIH consensus:

Category	Time of symptoms after HSCT	Presence of acute GVHD features	Presence of chronic GVHD features
Acute GVHD			
Classic acute GVHD	≤100 days	Yes	No
Persistent, recurrent or late-onset acute GVHD	>100 days	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

Chronic GVHD:

Chronic GVHD is a heterogeneous chronic syndrome,[3] that has emerged as the most troublesome complication of transplantation, as more patients survive the early post-transplant period due to improvements in HLA typing for unrelated transplants, better immunosuppressive prophylactic regimens, reduced intensity conditioning regimens, improved supportive care,[64] older recipient age and use of peripheral blood cells as graft source.[8] It is a pleiomorphic syndrome usually occurring around four months after the transplant,[69] between 3 and 24 months, resembling autoimmune diseases, and is associated with a reduced risk of recurrent malignancy.[8] Cutaneous GVHD can no longer be treated as a limited anti-epithelial process, but is also anti-endothelial and potentially anti-multicellular, resulting in cellular cytotoxicity and tissue damage.[1] Chronic GVHD can occur in three ways, in 20-30% patients, it presents as progression of acute GVHD known as the progressive type, in another 30-40% as recurrence after treatment of acute GVHD known as quiescent or interrupted type and in 35% without prior acute GVHD

known as the de novo type.[42,8] The de novo type has the least and the progressive type the highest mortality.[14] It can be triggered by UV radiation, trauma or infections like zoster or Borreliosis, or may appear spontaneously.[42] Cutaneous manifestations are seen in almost all patients with chronic GVHD,[14] in upto 80% of HLA identical transplants receiving Methotrexate prophylaxis with oral involvement in upto 72%.[8]

The extended clinical and histological spectrum of chronic GVHD includes lichenoid and sclerodermoid forms, the latter containing lichen sclerosus, morphea, panniculitis and fasciitis.[70] In chronic GVHD, the incidence of lichenoid GVHD, lichen planus-like, was found to be 9%, sclerodermoid GVHD was 38%, lichen sclerosus-like was 50% and eosinophilic fasciitis was 6%.[9] Sclerodermatous GVHD (Scl GVHD) comprises 10-15% of chronic GVHD.[27]

Early clinical features of chronic GVHD may be subtle, and include xerosis, keratosis pilaris-like lesions, ichthyosis, papulosquamous lesions, psoriasiform, pityriasis rosea-like lesions and annular lesions similar to annular psoriasis, subacute cutaneous lupus erythematosus or superficial erythema annulare centrifugum.[3] Hallmark features of chronic GVHD are dry, scaling skin[1] and poikiloderma.[3] Pigmentary changes include a bronzed hyperpigmentation,[38] leopard-like pigmentation,[9] vitiligo-like lesions,[38] leukoderma and leukotrichia.[71] Lesions resembling lupus erythematosus,[34] dermatomyositis,[72] a generalized ageing process,[38] reactive isomorphism,[73] exfoliative dermatitis[74] and erythema multiforme-like targetoid lesions have been described.[75] Guttate, shiny indurated lesions may be seen on the trunk.[34] Other features of chronic cutaneous GVHD include bullae or ulcers over sclerodermoid lesions, eczema craquele, nodular fibromas, cutaneous focal mucinosis,[9] eruptive angiomas,[76] hand and foot eczema-like vesiculation, scaling or acral keratoses, erythema annulare centrifugum,[65]

bullous pemphigoid, epidermolysis bullosa acquisita, histiocytic cytophagic panniculitis and pyoderma gangrenosum.[38] A newly described type of chronic cutaneous GVHD is termed eczematoid GVHD, which is a severe, usually erythrodermic eruption associated with a poor prognosis that requires substantial immunosuppression.[77] A maculopapular rash can occur in chronic GVHD, after reducing immunosuppressants or transfusing with donor lymphocyte infusions.[65] Planar xanthoma due to cholestasis secondary to chronic GVHD has been described.[78]

Scarring and non-scarring alopecia of scalp, axillary and pubic hair have been noted. Nail changes can occur early, and include periungual erythema, fragility, atrophy, onycholysis, thickening, longitudinal striations, dystrophy, pterygium, white patches[38] and periungual telangiectasias. Nail involvement was found in half the patients with chronic cutaneous GVHD in Turkey and was associated with longer duration of disease.[79] Changes in sweating may be present. Many patients, upto 80%, can have oral and genital epithelium involvement, including dryness, atrophy, hypertrophy, erosions and white plaques.[3] Oral pain was a common complaint in patients with chronic GVHD.[52] Oral involvement is seen in 72% patients with chronic GVHD,[8] mucosal involvement in upto 80% patients.[42] Mouth and eyes are the most common mucosal surfaces to be involved.[38] A Sjögren like syndrome is almost always seen.[42] Deep erythema is followed by reticulate white lichen planus-like mucosal lesions and hyperkeratotic plaques. Lesions may persist for many years, with a 4 times greater risk of squamous cell carcinoma than the general population.[38] Lingual papillae may be short, absent or even elongated, geographical tongue may be seen.[65] Of the oral manifestations, lichen planus-like features were the most distinctive, with the highest positive predictive value.[52] The incidence of ocular GVHD was found to be 5.5%, the most common presentation being keratoconjunctivitis

sicca.[80] On an average of 10 months post-transplant, vaginal symptoms, most commonly dryness, itching and tenderness develop, leading to dyspareunia.[81]

Lichen planus-like GVHD:

Lichenoid lesions were thought to be the early form of chronic cutaneous GVHD, but can occur before, with or after sclerodermoid change.[9] Classical lichen planus-like lesions are violaceous lichenoid papules and plaques seen over the dorsae of hands, forearms and trunk.[3,9] Common sites of involvement are the palms and soles, but groin, ears and peri-orbital region can be involved.[38] Perifollicular papules and follicular hyperkeratosis may be noted.[9] Folliculotropism may be a feature[3] and rarely, it may be dermatomal (kertinocyte antigenicity being altered by varicella zoster infection) or follow Blaschko's lines (cellular mosaicism causing a clone of cells to have different histocompatibility antigens).[38] Photosensitive eruptions similar to subacute cutaneous lupus erythematosus or dermatomyositis may represent lichenoid GVHD.[82]

Morphea-like GVHD:

Plaques of dermal sclerosis similar to morphea characterize the sclerodermoid type of chronic GVHD, which eventually progress to generalized scleroderma.[3] These lesions appear as circumscribed, firm, hyperpigmented or skin colored plaques that are indurated on palpation and favor the lower trunk,[9] major skin folds and proximal extremities.[38] A predilection for the limbs was recently described.[83] The skin appears 'hidebound' with 'pipestem legs'.[65] Other features include dermal nodules, atrophic white plaques and dimpled, hyperpigmented, tethered skin.[83]

Lichen sclerosus-like GVHD:

Lichen sclerosus-like features can be seen in genital or extragenital locations and comprise 50% of sclerodermatous patients. The neck and upper to mid-trunk, especially sites of recently removed central venous catheters are preferred sites. Hypopigmented plaques are seen with wrinkling, scaling and follicular plugging. The mean time of onset of these lesions was day 300, as an initial manifestation of sclerodermoid GVHD.[84] The link between lichenoid and sclerodermoid GVHD may be the lichen sclerosus lesions,[9] or these lesions may be a distinct subtype of chronic GVHD.[85]

Eosinophilic fasciitis-like GVHD:

Eosinophilic fasciitis-like features can be seen and represent a deep from of sclerodermoid GVHD. It can be seen in 58% patients, mean onset being day 700. Strenuous physical activity was found to precipitate this feature in many patients. Peripheral eosinophilia can be seen in upto 60% patients.[84] The limbs, excluding the hands and feet are most commonly involved. Acute pain and edema are followed by the appearance of induration known as "pseudocellulite". "Groove sign" refers to a depression along the course of a vein, between muscle groups, or both.[9] Deep fascial involvement is characterized by peau d'orange appearance. A positive prayer sign, where wrist dorsiflexion is acutely limited, may be seen.[65]

Signs and symptoms of chronic GVHD are modified from the NIH consensus in **Table8**[66].

Table 8. Signs and symptoms of chronic GVHD, NIH consensus:

Organ or site	Diagnostic criteria	Distinctive criteria	Other features	Common (seen with both acute and chronic GVHD)
Skin	Poikiloderma (atrophy and pigmentary changes) Lichen planus-like features Sclerotic features Morphea-like features Lichen sclerosus-like features	Depigmentation	Sweat impairment Ichthyosis Keratosis pilaris Hypopigmentation Hyperpigmentation	Erythema Maculopapular rash Pruritus
Mouth	Lichen-type features Hyperkeratotic plaques Restriction of mouth opening from sclerosis	Xerostomia Mucocele Mucosal atrophy Pseudomembranes Ulcers		Gingivitis Mucositis Erythema Pain
Genitalia	Lichen planus-like features Vaginal scarring or stenosis	Erosions Fissuring Ulceration		
Nails		Dystrophy Longitudinal ridging, splitting, brittle nails or loss of nails Onycholysis Pterygium unguis		
Scalp and body hair		New onset scarring or non-scarring alopecia Scaling, papulosquamous lesions	Thinning scalp hair, typically patchy, coarse, or dull (not explained by endocrine or other causes) Premature gray hair	
Eyes		New onset dry, gritty, or painful eyes Cicatricial conjunctivitis Keratoconjunctivitis sicca Confluent areas of punctuate keratopathy	Photophobia Periorbital hyperpigmentation Blepharitis	
GI tract	Esophageal web Strictures or stenosis in the upper to mid-third of the esophagus		Exocrine pancreatic insufficiency	Anorexia Nausea Vomiting Diarrhea

				Weight loss Failure to thrive (infants and children) Total bilirubin, alkaline phosphatase>2 x upper limit of normal
Lung	Biopsy showing bronchiolitits obliterans	Bronchiolitis obliterans diagnosed by pulmonary function testing or radiology		Bronchiolitis obliterans-organizing pneumonia
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to sclerosis	Myositis or polymyositis	Edema Muscle cramps Arthralgia or arthritis	
Hematopoietic and immune			Thrombocytopenia Eosinophilia Lymphopenia Hypo- or hypergammaglobulinemia Autoantibodies (AIHA and ITP)	
Other			Pericardial or pleural effusions Ascites Peripheral neuropathy Nephrotic syndrome Myasthenia gravis Cardiac conduction abnormality or cardiomyopathy	

If any of the manifestations included in the diagnostic criteria are present, the presence of chronic GVHD can be established without further testing or evidence of other organ involvement. Distinctive criteria are manifestations not ordinarily found in acute GVHD, but are not enough to establish the diagnosis of chronic GVHD without further testing. Other features can be a part of chronic GVHD, once the diagnosis is established. Common muco-cutaneous features can be seen in both acute and chronic GVHD.

Diagnosis of chronic GVHD can be made if there is at least one diagnostic manifestation or at least one distinctive manifestation with confirmation of diagnosis by biopsy or laboratory testing or imaging in the same or another organ.[66] Organ scoring of chronic GVHD, from the NIH consensus is summarized in **Table 9**.[9,66]

Table 9. Organ scoring of chronic GVHD, NIH consensus:

	Score 1	**Score 2**	**Score 3**
Skin	<18% BSA, no sclerotic features	19-50% BSA or superficial sclerosis	>50% BSA or deep sclerosis or impaired mobility, ulceration or severe pruritus
Mouth	Mild signs/symptoms not limiting oral intake	Moderate signs/symptoms with partial limitation of oral intake	Severe signs/symptoms with major limitation of oral intake
Eyes	Mild dry eye symptoms or asymptomatic but signs of keratoconjunctivitis sicca	Moderate dry eye symptoms partially affecting ADL, no visual impairment	Severe dry eye symptoms affecting ADL or unable to work or loss of vision
Gut	Symptoms without significant weight loss	Symptoms with weight loss of 5-15%	Symptoms with weight loss>15%, requiring nutritional supplementation or esophageal dilatation
Liver	Bilirubin, alkaline phosphatase or transaminases <2x of normal upper limit	All 2-5x of normal upper limit or bilirubin >3mg/dl	All >5x of upper normal limit
Lungs	Mild symptoms (SOB after 1 flight of steps), FEV_1 60-79% or LFS 2	Moderate symptoms (SOB after walking on flat ground), FEV_1 40-59% or LFS 6-9	Severe symptoms (SOB at rest), FEV_1 ≤39% or LFS 10-12
Joint/fascia	Mild tightness of arms or legs, mildly decreased ROM and not affecting ADL	Tightness of arms or legs, joint contractures, erythema due to fasciitis, moderately decreased ROM or mild to moderate limitation of ADL	Contractures with significantly decreased ROM and significant limitation of ADL
Genital tract	Mild signs/symptoms, no effect on coitus/minimal discomfort on examination	Moderate signs/symptoms and mild dyspareunia/discomfort on examination	Advanced signs (strictures, labial fusion or severe ulceration) and severe pain with coitus/ inability to insert vaginal speculum
Global assessment	Mild 1-2 organs, except the lung, with a maximum organ score of 1 each	Moderate ≥1 site with organ score 2 or ≥3 sites with an organ score of 1 or lung score of 1	Severe Any organ score of 3 or lung score of 2

ADL=Activities of daily living; BSA=body surface area; FEV_1=forced expiratory volume in 1 s; LFS=lung function score (includes FEV_1 and diffusion capacity of the lung for CO); ROM=range of motion; SOB=shortness of breath.

Cutaneous response to therapy can be graded using the NIH scale or the Hopkins scale.[86] Chronic GVHD was classified as limited or extensive, in **Table 10**.[87,34]

Table 10. Classification of chronic GVHD:

Limited chronic GVHD
Either or both:
1. Localized skin involvement
2. Hepatic dysfunction due to chronic GVHD
Extensive chronic GVHD
Either:
1. Generalized skin involvement, or
2. Localized skin involvement &/ or hepatic dysfunction due to chronic GVHD
Plus:
3a. Liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis, or
b. Involvement of eye (Schirmer test with<5mm wetting), or
c. Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or
d. Involvement of any other target organ

A revised classification was described in 2003, with features of clinical limited and clinical extensive chronic GVHD, depicted in **Table 11**.[8]

Table 11. Revised classification of chronic GVHD:

Clinical limited:
1. Oral abnormalities consistent with chronic GVHD, a positive skin or lip biopsy, and no other manifestations of chronic GVHD
2. Mild liver test abnormalities (alkaline phosphatase <2 x upper limit of normal, AST or ALT <3 x upper limit of normal, and total bilirubin <1.6) with positive skin or lip biopsy, and no other manifestations of chronic GVHD
3. Less than 6 papulosquamous plaques, macular-papular or lichenoid rash involving <20% of BSA, dyspigmentation involving <20% BSA, or erythema involving <50% BSA, positive skin biopsy, and no other manifestations of chronic GVHD
4. Ocular sicca (Schirmer's test <5 mm with no more than minimal ocular symptoms), positive skin or lip biopsy, and no other manifestations of chronic

GVHD

5. Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of chronic GVHD

Clinical extensive:

1. Involvement of 2 or more organs with symptoms or signs of chronic GVHD, with biopsy documentation of chronic GVHD in any organ
2. Karnofsky or Lansky Clinical Performance scores <60%, >15% weight loss, and recurrent infections not due to other causes, with biopsy documentation of chronic GVHD in any organ
3. Skin involvement more extensive than defined for clinical limited chronic GVHD, confirmed by biopsy
4. Scleroderma or morphea
5. Onycholysis or onychodystrophy thought to represent chronic GVHD, with documentation of chronic GVHD in any organ
6. Decreased range of motion in wrist or ankle extension due to fasciitis caused by chronic GVHD
7. Contractures thought to represent chronic GVHD
8. Bronchiolitis obliterans not due to other causes
9. Positive liver biopsy; or abnormal liver function tests not due to other causes with alkaline phosphatase >2 x upper limit of normal, AST or ALT >3 x upper limit of normal, or total bilirubin >1.6, and documentation of chronic GVHD in any organ
10. Positive upper or lower GI biopsy
11. Fasciitis or serositis thought to represent chronic GVHD and not due to other causes

Differential diagnosis:

There are no absolute clinical characteristics that can declare a rash to be due to a drug rather than GVHD.[88,69] Other differentials are viral infections, chronic radiodermatitis and autoimmune diseases like systemic lupus erythematosus, scleroderma, Sjögren's syndrome, rheumatoid arthritis, dermatomyositis and polymyositis. Oral candidiasis can mimic lichenoid GVHD.[34] In Scl GVHD, Raynaud's phenomenon and acrosclerosis are usually absent.[38,42]

Histopathology:

Acute GVHD:

The findings of a skin biopsy include an upper dermal infiltration by T cells, exocytosis into the epidermis and dyskeratotic epidermal cells.[59] Biopsy findings are characterized by the degree of keratinocyte damage and categorization is into 4 grades as in **Table 12**.[53,89]

Table 12. Histopathological grading of acute GVHD:

Histopathological grade	Features
1	Vacuolization of the basal keratinocytes
2	Dyskeratotic keratinocytes as well
3	Focal clefting of the basal layer
4	Separation of the epidermis from dermis totally

Apoptosis is a typical feature of GVHD. The dermo-epidermal junction is most severely affected.[15] Satellite cell necrosis is the term used to describe dyskeratotic keratinocytes in close proximity to epidermal lymphocytes.[90] To establish a diagnosis of GVHD, at least grade 2 changes should be present.[59] Grade 1 changes can be considered adequate in GVHD occurring after day 35.[91] No single feature on the biopsy can be considered pathognomonic.[9,92] The follicular epithelium can be an early target in acute cutaneous GVHD.[56] Histopathology of stage IV acute GVHD was classified as a necrolysis pattern, in which full epidermal necrosis seemed to originate in the upper layers, a GVHD pattern, in which necrosis of basal layers were noted at the edge of the

lesion representing vacuolar interface changes and a mixed necrolysis and GVHD pattern.[18]

The use of skin biopsy to confirm the diagnosis of acute cutaneous graft-versus-host disease is still controversial as delaying treatment of GVHD till a biopsy confirms the diagnosis could lead to rapid progression of the disease while on the other hand is the risk of sepsis with the use of immunosuppression.[58] The cause of a new onset rash in a transplant patient is best determined by close examination and follow-up of clinical features without a biopsy, biopsies seldom alter the treatment.[93] Treatment of suspected acute GVHD depends on clinical suspicion and not biopsy findings as they are mostly non-specific. A skin biopsy helps to consolidate the diagnosis with liver function tests and intestinal biopsy, gives insight into evolution to chronic GVHD and allows comparison with future skin biopsies.[58] Serial biopsies establish a baseline for interpretation of subsequent biopsies.[94] The diagnostic yield of a skin biopsy can be increased by careful selection of the site from an established perifollicular lesion by an experienced dermatologist.[9] Clinically involved skin can show unremarkable histology whereas normal skin can show features of GHVD.[30] Liver or intestine biopsy can unambiguously confirm the diagnosis of GVHD, unlike in the skin.[93]

In a retrospective study it was found that a skin biopsy performed before 21 days of the transplant had no value in ruling out acute GVHD and epidermal lymphocytes should be present to diagnose acute GVHD.[89] Another retrospective study determining the frequency and use of skin biopsy within 30 days of transplant revealed that biopsy features didn't correlate with clinical severity of the rash, which finally influenced the decision to treat the patient; hence the practice of performing a routine biopsy can be abandoned without comprising the care of the transplanted patient.[30] If the sensitivity and

specificity of the skin biopsy specimen is very high, the best clinical outcome is found when a skin biopsy is performed, while simultaneously initiating treatment, then treatment is revised according to results of the biopsy. Where the prevalence of GVHD is >30%, it is best to treat GVHD without a skin biopsy, whereas where the prevalence is <30%, a skin biopsy would best guide treatment.[58] The risk versus benefit ratio favors the performance of a biopsy of a suspected GVHD rash as this potentially life threatening complication needs aggressive immunosuppression.[9,95]

Differential diagnosis:

A skin biopsy, by suggesting another diagnosis may help to substantiate the clinical diagnosis, but can't prove the diagnosis of GVHD by itself.[15] Infectious processes can be suggested by a neutrophilic infiltrate, viral inclusions or special stains, while a drug rash can be suggested by abundance of eosinophils and chemotherapy induced changes by eccrine gland necrosis. Histologic changes may appear later than the clinical cutaneous eruption.[89] Histology of drug eruptions caused by drugs with a sulfhydryl group is similar to GVHD with liquefactive necrosis.[96] There are no reliable differentiating histological features between drug hypersensitivity and GVHD, hence it is better to presume GVHD and follow up the patient.[69] Chemotherapy artifact areas should be avoided while trying to establish a diagnosis of GVHD; they are defined by the loss of orderly progression of cuboidal basal cells to flat squamous cells, irregular nuclear contour and occasional dyskeratotic cells.[53] Acral erythema due to chemotherapy reveals vacuolar degeneration of the basal layer, necrotic keratinocytes, spongiosis, papillary dermal edema and a mild perivascular lympho-histiocytic infiltrate. Another histological differential includes subacute radiation dermatitis.[54] Biopsy findings of ELR include upper dermal infiltration of $CD3^{+}4^{+}$ lymphocytes, vascular dilatation, lymphocytic

exocytosis, intercellular edema and rarely, dyskeratotic keratinocytes.[53] Satellite cell necrosis can also be present in an ELR.[59]

Chronic GVHD:

Histopathological criteria for chronic muco-cutaneous GVHD, with clinical, laboratory and radiology yield four diagnostic categories namely, no GVHD, possible GVHD, consistent with GVHD and definite GVHD. The NIH consensus criteria are shown in **Table13**.[92]

Table13. NIH consensus criteria for chronic GVHD:

Organ or system	**Minimal criteria for active GVHD**	**Specific criteria for chronic GVHD**
Skin, any stage	Apoptosis in the epidermal basal layer or lower malpighian layer or outer root sheath of hair follicle or acrosyringium ± lichenoid inflammation ± vacuolar change ± lymphocytic satellitosis	
Skin, lichen planus-like		Combination of epidermal orthokeratosis, hypergranulosis and acanthosis with lichenoid changes ± syringitis of eccrine units ± panniculitis
Skin sclerotic		Collagenous deposition with thickening throughout the papillary dermis, or pan-dermal collagenosis ± panniculitis
Skin morpheic		Clinically focal or localized lesions predominated by sclerosis in the lower reticular dermis or along the dermal-hypodermal border ± epidermal and appendageal involvement

Skin fasciitis		Fibrous thickening of fascial septa with adjacent inflammation ± panniculitis
Liver	Global assessment of dysmorphic or destroyed small bile ducts ± cholestasis, lobular &/or portal inflammation	Ductopenia, portal fibrosis, and chronic cholestasis reflect chronicity but are not specific for chronic GVHD
Gastrointestinal	Variable apoptotic criteria (≥1/piece) in crypts	Destruction of glands, ulceration, or submucosal fibrosis reflects long-standing disease but are not specific for chronic GVHD
Oral mucosa and conjunctiva	Lymphocytic infiltration of mucosa with variable apoptosis	
Minor salivary or lacrimal gland		Infiltration and damaged intralobular ducts, fibroplasias in periductal stroma, and inflammation with destruction of acinar tissue
Lung		Obliterative bronchiolitis: dense eosinophilic scarring beneath the respiratory epithelium, resulting in complete fibrous obliteration or some degree of luminal narrowing

GVHD Prophylaxis:

The main approach for prevention of GVHD is to avoid the risk factors if possible. Strategies to prevent GVHD include matching of histocompatibility antigens of the host and donor, in vivo immunosuppression as prophylaxis, protective environment, gut decontamination, total lymphoid irradiation, thymic transplant and in vitro treatment of donor stem cells, like ATG with complement, monoclonal anti-T cell antibodies, E-rosette depletion, lectin separation, elutriation and immunoadsorbent column.[52] Without prophylaxis, incidence of GVHD can approach 100%.[5] An ideal prophylactic regimen would eliminate acute and chronic GVHD, allow immunologic recovery effectively and maintain graft-versus-tumor effect.[97] Calcineurin inhibitors, with or without Methotrexate

are used as immunosuppressive drugs. Manipulation of the graft by depleting the T cells has lower organ toxicity than immunosuppressants but is associated with increased risk of relapse or rejection, delayed immune reconstitution, decreased functional recovery of T lymphocytes and impaired recovery of diversity of the T cell repertoire.[3] Standard GVHD prophylactic regimen target the second phase of the pathophysiology of GVHD, calcineurin inhibitors inhibit T cell proliferation and IL-2 expression, whereas Methotrexate targets T cells that are rapidly dividing.[98]

A recent meta-analysis done to evaluate various prophylactic regimens for acute GVHD concluded that the combination of Cyclosporine and Methotrexate was superior to Cyclosporine and that the combination of Methotrexate and Tacrolimus was superior to Methotrexate and Cyclosporine with respect to lowering acute GVHD.[99]

In randomized trials aimed at chronic GVHD prevention, there was no difference in incidence of chronic GVHD irrespective of the dose or duration of Cyclosporine, 6 months versus 24 months,[7] or with addition of immunoglobulin irrespective of dose.[8]

Combination drug prophylaxis is usually given immediately post-transplant which is slowly tapered after 100 days and stopped around 180 days. Tacrolimus was found to be as effective as Cyclosporine but less toxic, but patients on Cyclosporine had a better survival.[5]

New agents for GVHD prophylaxis include Rapamycin, Trimetrexate, Deoxyspergualin, Chloroquine, PG27, CTLA4Ig, Glutamic acid-lysine-alanine-tyrosine (GLAT), Neuraminidase, Fludarabine and 2 deoxy-chloroadenosine.[5] Cytokine based approaches include antithymocyte globulin, anti-TNFα targeted strategies (Infliximab, Etanercept) and anti-IL-2 receptor antibody (Daclizumab).[3]

Treatment of GVHD:

GVHD is still considered the "Achilles heel" of HSCT.[97]

Acute GVHD:

The standard of treatment for acute GVHD is corticosteroids, the mechanism of action probably due to lympholytic action. Methylprednisolone 2mg/kg/day is the starting dose.[5] If after 3 days, the disease worsens, after 7 days remains the same or after 14 days doesn't respond fully; second line therapy should be initiated.[46] Other therapies include Thalidomide,[3] Hydroxychloroquine, Methotrexate, Acitretin, Cyclophosphamide,[9] monoclonal antibodies for treatment of acute GVHD, including Zomazyme, B-C7, IL-1 receptor antagonist, CD11a antibody[5] and Rituximab.[9] Other skin directed therapies include phototherapy and photochemotherapy; UVA1 (340-400nm) is especially effective in sclerodermoid GVHD.[9] Supportive care is as important as targeted therapy.[1] Therapeutics in steroid resistant acute GVHD are summarized in **Table 14**.[100]

Table 14. Therapeutics in steroid resistant acute GVHD:

Polyclonal antibody	Antithymocyte globulin
Monoclonal antibody	OKT3, Visilizumab, ABX-CBL, Daclizumab, Inolimomab, Basiliximab, Alemtuzumab, Alefacept
Biologic toxin-conjugate	Denileukin diftitox
TNFα blocker	Infliximab, Etanercept
Chemotherapy	Mycophenolate, Pentostatin, calcineurin inhibitors, Sirolimus
Phototherapy	PUVA, photochemotherapy
Cellular therapy	Mesenchymal stem cells
Topical / directed therapy	Oral Beclomethasone or Budesonide for intestinal GVHD; intra-arterial steroid or Methotrexate infusion

Chronic GVHD:

The principles of management of chronic GVHD include patient education,[8] immunosuppression, topical therapy and antibiotic prophylaxis for infection.[101] Ongoing supportive care, adequate hydration, maintenance of nutrition and careful follow up are essential.[7] Strict vigilance against complications of the disease and treatment are indicated, including infections, hypertension, hyperglycemia, osteoporosis, renal dysfunction and hyperlipidemia.[102] Drugs used to treat acute GVHD are also useful to treat chronic GVHD.[103] At diagnosis, Prednisolone 1mg/kg/day with Cyclosporine 10mg/kg/day is given. Tapering is by 25% per week after two weeks, if the disease doesn't worsen till 1mg/kg prednisolone on alternate days. After steroid tapering, Cyclosporine is tapered by 25% per week, alternating with Prednisolone. Most patients show response to therapy in three months.[101] Most patients require prolonged steroid therapy; only less than half are able to discontinue immunosuppression by two years.[102] If there is failure to improve after at least two months of treatment, or worsening after a month of standard therapy, including steroids and Cyclosporine, it is referred to as steroid refractory chronic GVHD.[8] There is no standard salvage therapy for these patients, in small phase II trials and case series, various agents have been tested, and have response rates of 20-80%.[102]

For local therapy of chronic GVHD, topical steroids have been the standard treatment. Topical Tacrolimus ointment has been used as a safe alternative with transient burning being the only major side effect, and serum levels being below the systemic therapeutic range for graft rejection prevention. The control of erythema and pruritus was evident rather rapidly.[104]

Prognosis:

Acute GVHD:

Prognosis of acute GVHD correlates with the initial stage at presentation. Long term survival of patients with acute GVHD grade 0-I is about 50% and grade IV is upto 11%.[100] Acute GVHD is the reason for death in 40 to 50% patients with moderate to severe disease.[46,52] Severity of acute GVHD is the most important prognostic factor predicting the outcome of the transplant.[30] Mild to moderate acute GVHD (grade I or II, A or B) has less mortality, but is a risk factor for development of subsequent chronic GVHD, whereas severe GVHD (grade III or IV, C or D) has a grave prognosis with a mortality rate as high as 100%.[15] Patients who survive grade IV acute GVHD develop severe chronic GVHD less often.[105] The onset pattern of late acute GVHD symptoms and not presence of features of acute GVHD may be important in prognosis.[68] The most predictive parameter for progression of GVHD is the peak clinical stage.[106]

Chronic GVHD:

The presence of chronic GVHD is considered the main determinant of late infectious complications.[38] It is the main cause of non-relapse mortality in transplant survivors and has a major impact on quality of life and functional status of these patients.[107] It is associated with 30-50% risk of mortality.[8] Prognostication in chronic GVHD includes timely diagnosis; appropriate management and close follow up. Non-relapse mortality in patients with chronic GVHD was related to extensive skin involvement, progressive onset of chronic GVHD from acute GVHD and thrombocytopenia.[101] A scheme for grading chronic GVHD was suggested based on the prognostic factor score and absolute number of risk factors, which can be used to individualize treatment of patients.[101] GVHD is the cause of death in 12 to 20% patients. Death in these patients is usually due to infection, cachexia or liver failure.[42] Most deaths are due to infection.[101] Ten year survival rate is 80% in patients with mild chronic GVHD

and 5% in those with severe chronic GVHD.[34] Skin involvement in sclerodermoid GVHD indicates better disease-free survival due to decreased relapse rate, but extracutaneous involvement increases mortality rate.[83] Many reports suggest that lichenoid GVHD histology is a poor prognostic factor,[108,109] whereas sclerodermatous histology is not, but Akpek et al[110] found that histology of cutaneous chronic GVHD didn't have any prognostic value. A retrospective study revealed that the three year survival of patients with late acute GVHD was worse than overlap syndrome and chronic GVHD.[67] Despite treatment, the outcome of a third of patients with chronic GVHD remains the same.[101]

MATERIALS AND METHODS

Study design:

Single-centre, prospective, longitudinal cohort study

Study setting:

This study was conducted in Christian Medical College, an 1800 bedded tertiary care institution in Vellore, Tamil Nadu, after approval by the institutional review board. The patients were recruited from the Haematology out-patient department and wards. The first bone marrow transplant in this centre was done in 1986. Bone marrow transplants have been performed regularly and successfully since 1988. Peripheral blood stem cell transplants began in 1998. The first cord blood transplant was done in 2001 and the first unrelated donor transplant in 2008.

Study subjects:

The patient population eligible to be in the study was all patients in the Hematology department undergoing a stem cell transplant.

Inclusion criteria:

All patients undergoing allogeneic hematopoietic stem cell transplantation and consented to participate in the study.

Exclusion criteria:

Patients who were not willing for the study.

Study period:

The study period was from March 2009 to July 2010; patients were recruited from March, 2009 to April, 2010 and followed up till July, 2010.

Methodology:

All patients undergoing hematopoietic stem cell transplantation were screened and recruited into the study prior to the transplant procedure. At the time of registration into the study, a written informed consent was taken from the patient (Annexure 1), or the parent or legally acceptable guardian if the patient was a minor. A children's assent form (Annexure 2) was used for minors who were literate. All details of history, clinical examination and relevant investigations were recorded in a proforma (Annexure 3).

All patients were examined by the principal investigator (PI) prior to the transplant to enable post transplant changes to be recognized clearly. Demographic data was collected from the records, as were the pre-transplant diagnosis, donor related information, stem cell source and conditioning regimen. Any skin lesion present prior to the transplant was recorded. A detailed dermatological evaluation was done including the skin, nail and mucosal surfaces. The day the stem cells were infused was termed 'Day 0'. During the transplant period the patient was admitted in the Bone Marrow Transplant Unit (BMTU) and followed up daily. Engraftment was considered as absolute neutrophil count of ≥500/mm3 (0.5×10^9/L) achieved for three consecutive laboratory values tested on different days and platelets of $\geq 20 \times 10^9$/L achieved for three consecutive laboratory values, with no platelet transfusions in the previous seven days. If the patient developed any skin rash, this was evaluated by the PI. The presence and extent of skin rash and oral mucosal involvement was clinically assessed, photographs were taken and biopsies performed where indicated, to help confirm the diagnosis, with the patient's consent. A 4mm punch biopsy specimen was taken, which was fixed in skin fixative solution;

histopathological examination by light microscopy with haematoxylin and eosin staining was done by the department of Pathology. The patient was also monitored for any occurrence of diarrhea to evaluate for gut GVHD and abnormalities in liver function tests to evaluate for liver GVHD. Once the patient stabilized and was discharged, follow up was periodically thereafter, every week or month, or if any muco-cutaneous symptoms appeared. Any cutaneous clinical feature consistent with GVHD which appeared before 100 days post-transplant was considered as acute GVHD, and with onset after 100 days was considered chronic GVHD. Acute GVHD was graded clinically as per the1994 Consensus Conference, summarized in **Table 4**.[62,60,61] A biopsy was done for any new onset rash, unless the patient declined consent for the same, or the patient had characteristic white reticulate oral mucosal plaques occurring after 100 days post-transplant clinically suggestive of lichenoid GVHD. If chronic GVHD occurred as a continuation of acute GVHD, it was classified as progressive, if it occurred after complete resolution of acute GVHD, as quiescent and if it occurred without prior acute GVHD as de novo. Chronic GVHD was further classified as limited or extensive, as in **Table 10**.[87,34] Histopathological grading was done as in **Table 12**[53,89] and the NIH consensus criteria for chronic GVHD was used, as shown in **Table 13**.[92] Patients with significant gastrointestinal symptoms were subjected to a biopsy to confirm the clinical diagnosis of GVHD. Liver biopsy was not done; the diagnosis of liver GVHD was purely clinical and depended on liver function testing. Routine investigations, including total count, differential count and liver function tests were done as per the standard protocol of transplant in the Haematology department, daily till the patients engrafted, then on alternate days for a week, then twice a week till the patient was discharged, thereafter at follow up, once a month or once in 3 months as required, or earlier if there were any abnormal results.

Research committee approval:

The Institutional Review Board approved of this study (Annexure 4).

Statistical analysis:

The sample size was 92. Sample size was calculated based on the prevalence of GVHD found to be 35.7% in thalassemia patients in India, a precision of 10% with confidence intervals of 95%.

$$N = \frac{4 \times p \times q}{d^2} = \frac{4 \times 36 \times 64}{10^2} = 92$$

where, p= prevalence of GVHD, q= p-1 and d is the difference.

All statistical analysis was performed using SPSS 11.0 for windows (SPSS Inc, Chicago, IL). Frequency and percentages were used to describe the distribution of categorical variables, median and ranges were used to describe continuous variable that were not normally distributed. Chi-square test was used to assess the association between categorical variables. Continuous variables were compared using t-test. Weighted kappa statistic was used to assess agreement between clinical and histopathological grades of GVHD; squared weights were employed. The agreement was analyzed using R 2.8.0 ("irr" library). Logistic regression was employed to estimate the adjusted odds ratios as measure of risk for GVHD against potential risk factors. Kaplan Meier plots were used to display the distribution of times to engraftment and GVHD.

A p-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic profile:

Of a total of 105 patients who underwent allogeneic hematopoietic stem cell transplantation during the study period, 102 consecutive patients who consented were recruited into the study.

The age and sex distribution of the recipients is represented in **Table 15**.

Table 15. Age and sex distribution of the transplant recipients:

Age group in years	Males (%)	Females (%)
<10	19 (59.4)	13 (40.6)
11-20	13 (68.4)	6 (31.6)
21-30	12 (75)	4 (25)
31-40	12 (75)	4 (25)
41-50	8 (61.5)	5 (38.5)
51-60	3 (50)	3 (50)

Socio-demographic profile of the patients and donors is summarized in **Table 16**.

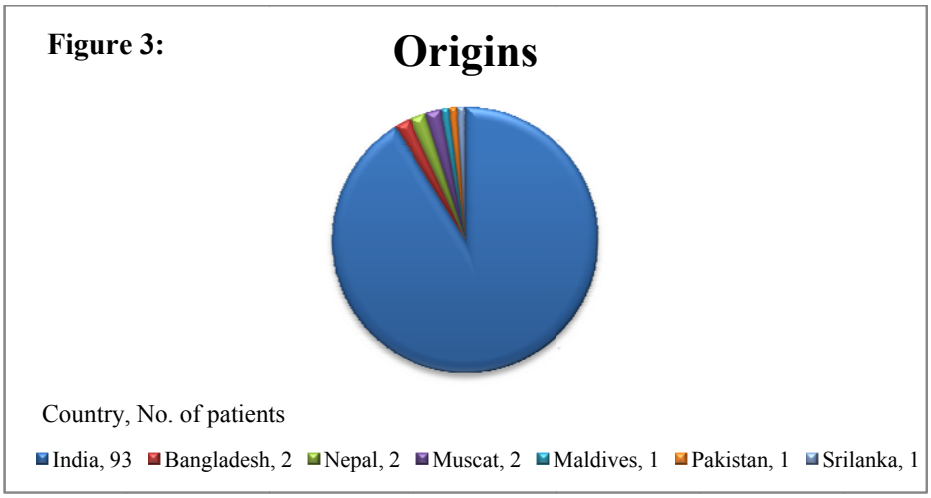
Table 16. Socio-demographic profile:

Variable	Descriptive statistics
Patient age*	20.5 (1-58)
Children (<15 years)*	44 (43.1)
Patient gender:	
Males#	67 (65.7)
Females#	35 (34.3)
Marital status:	
Married#	40 (39.2)
Urban residence#	90 (88.2)
Donor age*	26 (2-60)
Donor gender:	
Males#	57 (55.9)
Females#	45 (44.1)

*Median (range) #Frequency (%)

The recipient population consisted of Indians and few foreign nationals, shown in **Figure 3**.

Figure 3:

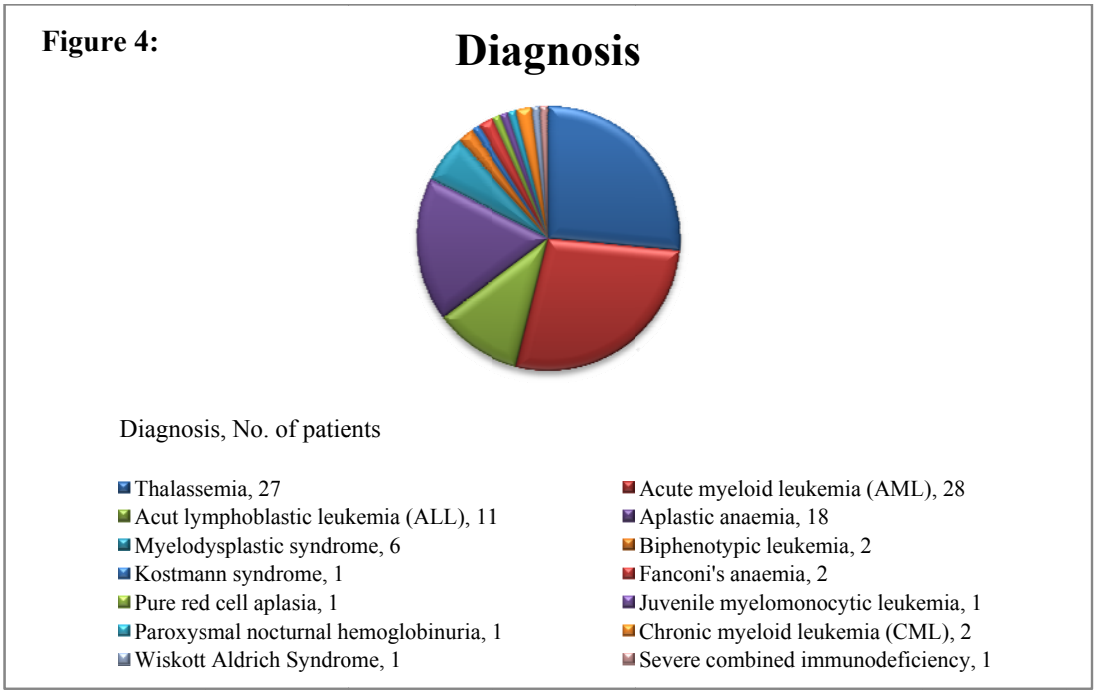


Majority of the patients were Indians, 91.2%.

<u>Patient diagnosis profile</u>:

The primary diagnoses of the patients are summarized in **Figure 4** below.

Figure 4:



The most common diagnoses were AML, thalassemia, aplastic anemia and ALL.

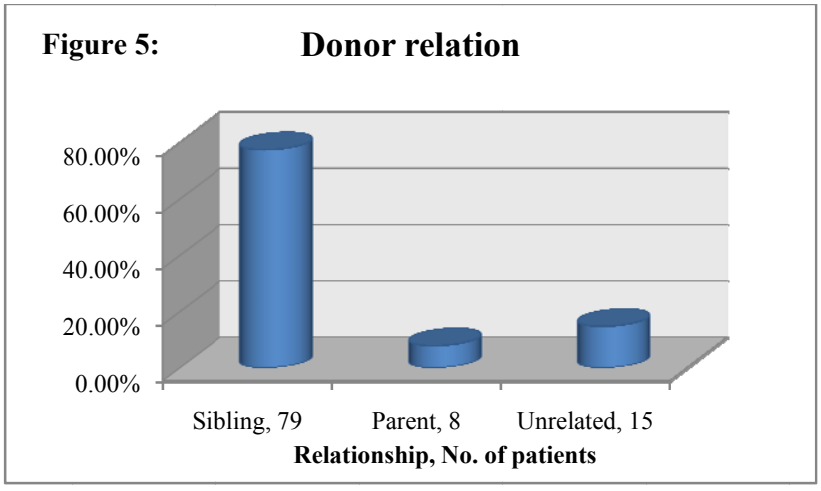
Past medical history:

Ninety nine patients (97.1%) had received prior blood transfusions.

<u>Donor compatibility profile</u>:

Relationship of the donor:

Figure 5 depicts the relationship of the donor with the recipient. Siblings were the most common donors, 79 (77.5%); unrelated donors were 15 (14.7%).



Distribution of patients according to the primary diagnosis and donor relation is depicted in **Table 17**.

Table 17. Primary diagnosis and donor relation:

Diagnosis	Relationship of the donor		
	Sibling	**Parent**	**Unrelated**
Thalassemia	24 (88.9%)	3 (11.1%)	-
AML	19 (67.9%)	2 (7.1%)	7 (25%)
ALL	8 (72.7%)	-	3 (27.3%)
Aplastic anemia	16 (88.9%)	2 (11.1%)	-
Myelodysplastic syndrome	3 (50%)	-	3 (50%)
Biphenotypic leukemia	1 (50%)	-	1 (50%)
Kostmann syndrome	1 (100%)	-	-
Fanconi's anemia	2 (100%)	-	-
Pure red cell aplasia	1 (100%)	-	-
Juvenile myelomonocytic leukemia	1 (100%)	-	-
Paroxysmal nocturnal hemoglobinuria	-	-	1 (100%)
CML	2 (100%)	-	-
Wiskott Aldrich Syndrome	1 (100%)	-	-
Severe combined immunodeficiency	-	1 (100%)	-

Unrelated donor transplants were done for AML, ALL, myelodysplastic syndrome, biphenotypic leukemia and paroxysmal nocturnal hemoglobinuria.

HLA typing:

High resolution typing was done for unrelated transplants, 10/10 antigens being HLA matched, whereas low resolution typing was done for related transplants, identical HLA type being 6/6. Most of the related transplants were HLA matched whereas the unrelated transplants mostly had a single antigen mismatched. HLA typing is summarized in **Table 18**.

Table 18. HLA typing of related and unrelated transplants:

HLA type	Related (%)	Unrelated(%)
Identical	80 (92)	3 (20)
Mismatch of 1 antigen	5 (5.7)	9 (60)
Mismatch of >1 antigen	2 (22.9)	3 (30)

Blood grouping:

ABO blood group incompatibility was seen in 46 patients (45.1%).

Gender mismatch:

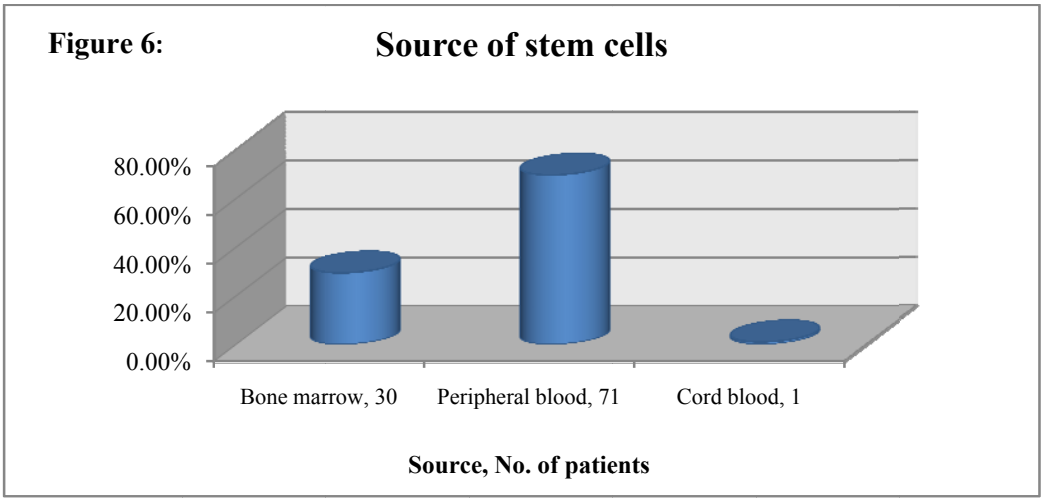
Gender mismatch from a male donor to female recipient was 24 (23.5%), and female donor to male recipient was 34 (33.3%). Female donors who had been pregnant in the past and donated to male recipients were 13 (38.2%).

Transplantation profile:

Source of hematopoietic stem cells:

The source of stem cells was bone marrow, peripheral blood or cord blood, as shown in **Figure 6** below.

Figure 6:



The most common source of hematopoietic stem cells was peripheral blood in 71 patients (69.6%), followed by bone marrow in 30 patients (29.4%).

Distribution of patients according to primary diagnosis and source of hematopoietic stem cells is depicted in **Table 19**.

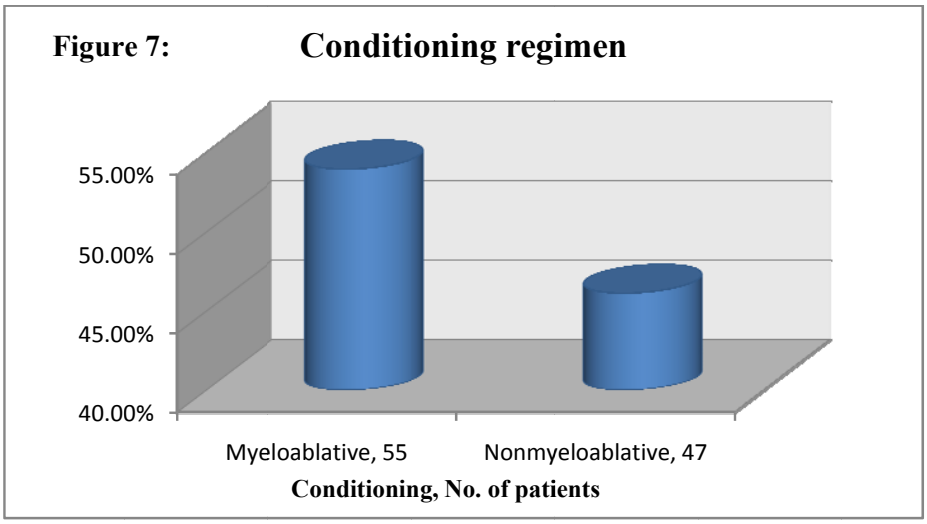
Table 19. Primary diagnosis and source of stem cells:

Diagnosis	Source of hematopoietic stem cells		
	BMT	**PBSCT**	**CBT**
Thalassemia	25 (92.6%)	2 (7.4%)	-
AML	-	28 (100%)	-
ALL	-	11 (100%)	-
Aplastic anemia	-	18 (100%)	-
Myelodysplastic syndrome	1 (16.7%)	5 (83.3%)	-
Biphenotypic leukemia	-	2 (100%)	-
Kostmann syndrome	1 (100%)	-	-
Fanconi's anemia	1 (50%)	1 (50%)	-
Pure red cell aplasia	1 (100%)	-	-
Juvenile myelomonocytic leukemia	-	1 (100%)	-
Paroxysmal nocturnal hemoglobinuria	-	1 (100%)	-
CML	-	2 (100%)	-
Wiskott Aldrich Syndrome	-	-	1 (100%)
Severe combined immunodeficiency	1 (100%)	-	-

Peripheral blood has become the most common source of stem cells, although bone marrow transplants are still being done, mostly for the thalassemia patients. Cord blood transplantation was performed in a single patient with Wiskott Aldrich Syndrome.

Conditioning regimen:

The conditioning regimens were grouped as myeloablative, in 55 patients (53.9%) and non-myeloablative in 47 patients (46.1%), shown in **Figure 7**.



Of the myeloablative conditioning, the most commonly used regimens were Thiotepa, Treosulphan and Fludarabine, in 9 patients (16.4%) and TBI and Cyclophosphamide in 5 patients (9%). Of the non-myeloablative conditioning, the regimens used most often were Busulphan, Cyclophosphamide and ATG in 16 patients (34%) and Fludarabine and Melphalan in 13 patients (27.7%).

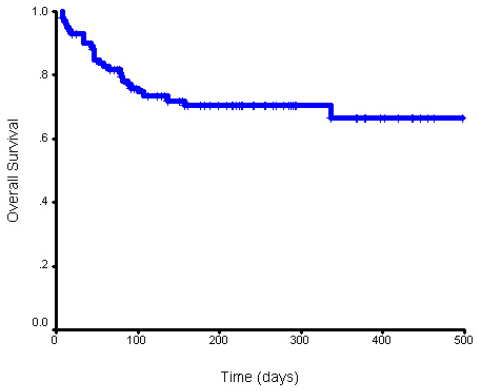
GVHD prophylaxis:

For GVHD prophylaxis, Cyclosporine and Methotrexate was given most often, in 96 patients (94.1%). In the other 6 patients (5.9%), regimes were Cyclosporine alone, 3 patients (2.9%), Methotrexate alone, Methotrexate and Tacrolimus, and Cyclosporine and Mycophenolate in 1 patient each.

Follow up period:

The median follow up was 164 days, range 8-515 days. The overall survival of patients was plotted as a Kaplan Meier curve in **Figure 8**.

Figure 8. Overall survival:



Engraftment:

The median time to engraftment of neutrophils was 16 days (range, 9-57), depicted in **Figure 9** and time to engraftment of platelets was 17 days (range, 8-61), depicted in **Figure 10**. Seven patients expired prior to engraftment (6.9%) and were not evaluable for GVHD.

Figure 9. Time to neutrophil engraftment:

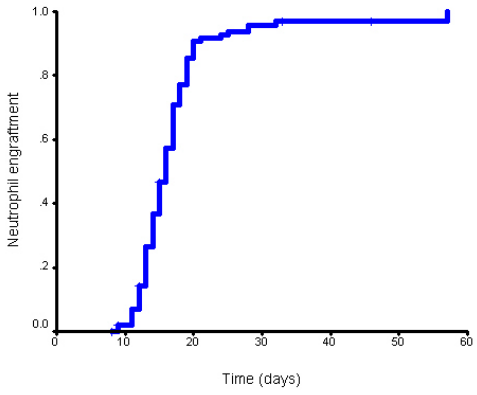
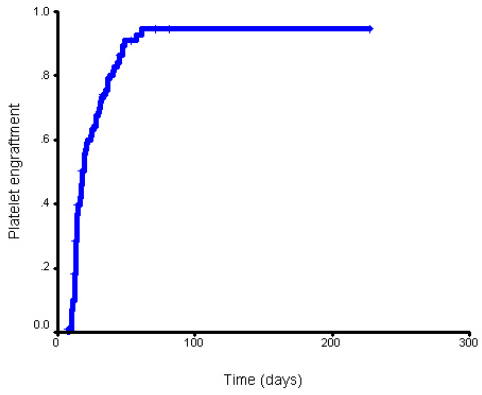


Figure 10. Time to platelet engraftment:



Graft versus host disease:

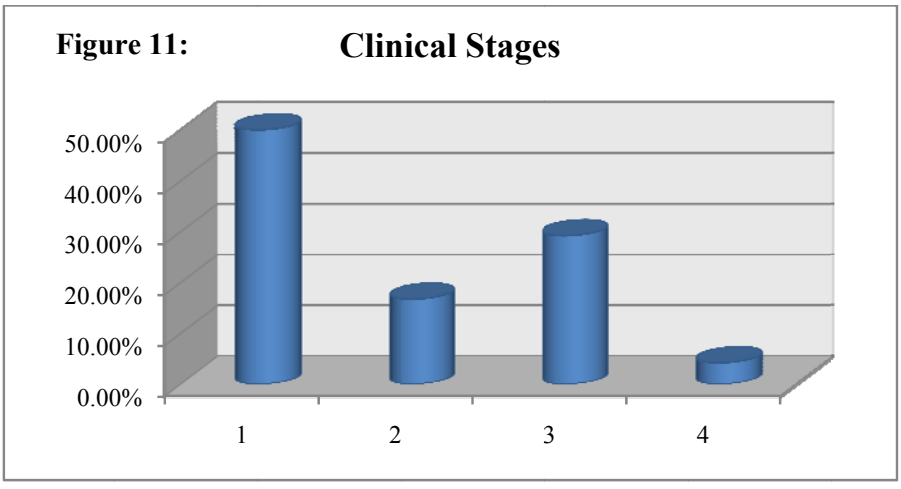
Hyperacute GVHD:

Hyperacute GVHD was seen in a single patient with only skin involvement, she had a maculopapular rash on day 11; neutrophil engraftment was on day 15 and platelet engraftment on day 13. Histology was consistent with acute GVHD, grade 2.

Acute GVHD:

Acute muco-cutaneous GVHD, as defined by the onset of muco-cutaneous clinical features of GVHD prior to 100 days post transplant, was seen in 25 patients (26.3%), of which one had only mucosal involvement, over the glans penis (4%). In the other 24 patients, and including the patient with hyperacute GVHD, the clinical stages are shown in **Figure 11**.

Figure 11:



Of the patients with acute cutaneous GVHD, stage 1 was seen in half the patients. Severe acute cutaneous GVHD, stages 3 and 4, was seen in 33.3%. Clinical presentation of cutaneous features of acute GVHD is described in **Table 20**.

Table 20. Patterns of cutaneous involvement in acute GVHD:

Cutaneous Involvement	No. of patients
Maculo-papular rash	14
Follicular GVHD	1
Flexural involvement	1
Dyspigmentation	1

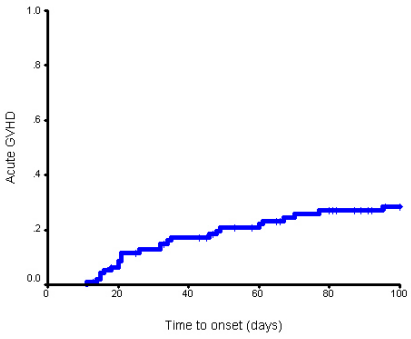
Oral mucosal involvement was always white reticulate plaques similar to lichen planus. Involvement of the glans penis was seen in 2 patients, 1 had erythema and erosions and the other had violaceous plaques similar to lichen planus, along with oral mucosal and lip involvement.

Of the 15 unrelated donor transplants, acute muco-cutaneous GVHD was seen in 5 patients (33.3%), of the sibling donor transplants, 18 patients (22.8%) and of the parent donors, 2 patients (25%).

Of the BMTs, 6 patients (20%) had acute muco-cutaneous GVHD and of the PBSCTs, 19 patients (26.8%) had acute muco-cutaneous GVHD. The patient with CBT did not develop GVHD.

Median time to onset of acute muco-cutaneous GVHD was 32 days (range, 11 to 95), depicted in **Figure 12**.

Figure 12. Time to onset of acute GVHD:



Acute gastrointestinal GVHD was seen in 35 patients (36.8%), and the histopathology was consistent with the diagnosis of GVHD. Acute liver GVHD was seen in 29 patients (30.5%).

Totally, 52 patients (50.9%) had acute GVHD.

Chronic GVHD:

Chronic GVHD, as defined by the onset of clinical features of GVHD after 100 days post transplant, was evaluated in 61 patients who followed up for >100 days.

Fifteen patients (24.6%) had chronic muco-cutaneous GVHD. Cutaneous involvement was mostly seen as violaceous plaques over the lips, face and body, a single patient had reticulate hyperpigmented plaques over the palms and soles, 1 patient had vitiliginous GVHD and 2 patients had a maculopapular rash. Involvement of the glans was seen in only 1 patient, who had white reticulate plaques. Nail involvement was seen in 1 patient. Sclerodermoid GVHD was not seen.

Clinical presentations of chronic muco-cutaneous GVHD are summarized below in **Table21**.

Table 21. Patterns of muco-cutaneous involvement in chronic GVHD:

Morphological type of presentation	Number	%
Lichenoid:		
1. Skin	8	53.3%
2. Oral	10	66.7%
3. Genitals	1	6.7%
4. Nails	1	6.7%
Sclerodermatous:	-	-

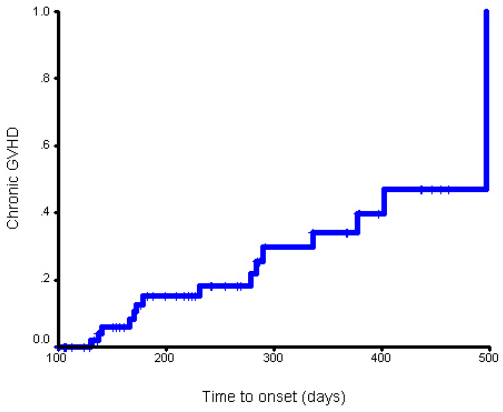
Others: 1. Maculopapular rash 2. Vitiliginous GVHD	2 1	13.3% 6.7%

Of the 15 unrelated donor transplants, chronic muco-cutaneous GVHD was seen in 2 patients (13.3%), of the sibling donor transplants, 11 patients (13.9%) and of the parent donors, 2 (25%).

Of the BMTs, none developed chronic muco-cutaneous GVHD whereas of the PBSCTs, 15 patients (21.1%) did. The patient with CBT did not develop GVHD.

Median time to onset of chronic muco-cutaneous GVHD was 231 days (range, 130 to 497) shown in **Figure 13**.

Figure 13, Time to onset of chronic GVHD:



Prior acute muco-cutaneous GVHD was seen in 3 patients (20%) with chronic muco-cutaneous GVHD. Prior acute gut GVHD was seen in 9 of these patients (60%) and prior liver GVHD in 6 (40%) of them.

Chronic gastrointestinal GVHD was seen in 2 patients (3.3%), both had prior acute gut GVHD, 1 had prior acute muco-cutaneous GVHD and the other had prior acute liver GVHD. Chronic liver GVHD was seen in 2 patients (3.3%), both had prior acute gut GVHD and 1 had prior acute muco-cutaneous GVHD.

Totally, 17 patients had chronic GVHD, of whom 13 had a history of prior acute GVHD.

Clinical pattern of chronic GVHD based on presence of prior acute GVHD is summarized in **Table 22**.

Table 22. Clinical pattern of chronic GVHD:

	Progressive	**Quiescent**	**De novo**	**Total**
Number	1	12	4	17
%	5.8	70.6	23.5	100

Chronic GVHD was further classified on the basis of extent of involvement. Extensive chronic GVHD was seen in 2 patients, and limited chronic GVHD was seen in all the other patients.

The incidence of muco-cutaneous GVHD, both acute and chronic, was 37 out of 95 patients (38.9%). Gastrointestinal GVHD was seen in 35 patients (36.8%), liver GVHD in 31 patients (32.6%). Totally, 56 patients had GVHD (58.9%).

Histopathology:

Skin biopsies were performed in 37 patients, of which 7 patients had 2 or more biopsies. A total number of 48 biopsy specimens were taken to rule out GVHD. Skin biopsies were consistent with GVHD in 31 patients. Out of 38 biopsies which showed

acute GVHD, 7 had grade 1 changes, 20 had grade 2, 11 had grade 3 and none had grade 4 changes. A single patient had features of chronic lichenoid GVHD. In 2 patients, during the same episode, 2 sites were biopsied and 1 site showed GVHD. In another 3 patients, both sites showed GVHD, of differing grades. In 3 patients, the biopsy was not consistent with GVHD. One patient had 4 biopsies at different times, 3 of which were done to rule out GVHD; 2 were consistent with GVHD, grades 1 and 3 in chronological order.

Clinico-histopathological correlation was done for acute cutaneous GVHD. If at the same time, with the same clinical stage, 2 histopathological grades from 2 biopsy samples were present, the higher grade was used. Level of agreement between clinical cutaneous grade and histological grade was checked in 27 biopsies, it was found that there was a negative agreement between the grades (Kappa=-0.169, p= 0.325).

Table 23 enlists the clinical and histological grades.

Table 23. Clinical and histological grades of acute GVHD:

Clinical grade	**Histological grade**			
	1	**2**	**3**	**4**
1	-	10	4	-
2	1	2	1	-
3	4	1	4	-
4	-	-	-	-

<u>Risk factors for GVHD</u>:

The following risk factors were analyzed for acute and chronic GVHD in **Table 24** and **25** respectively.

Table 24. Risk factors for acute GVHD:

Variable	Risk	95% Confidence intervals	p-value
Patient age <15 years	1		
Patient age ≥15 years	5.600	2.306 - 13.600	0.000
Donor age <15 years	1		
Donor age ≥15 years	5.494	2.156 - 14.003	0.000
Source of hematopoietic stem cells: Bone marrow	1		
Peripheral blood	3.947	1.540-10.113	0.004
Gender matched or male donor to female recipient	1		
Female donor to male recipient	1.220	0.523-2.847	0.646
ABO incompatibility	1.002	0.444-2.260	0.997
Donor relation: sibling	1		
Parents	2.000	0.345-11.578	0.439
Unrelated	3.333	0.850-13.069	0.084
Nonmyeloablative conditioning	1		
Myeloablative Conditioning	0.720	0.319-1.625	0.429
Related, HLA matched	1		
Mismatched	2.000	0.345- 11.576	0.439
Unrelated, HLA matched	1		
Mismatched	4.500	0.190- 106.813	0.352

Increasing patient age and donor age were found to be statistically significant risk factors for acute GVHD; the risk was about 5 times higher if the age was more than 15 years. If the source of stem cells was peripheral blood compared to bone marrow, after excluding the single patient with cord blood transplantation, the risk of acute GVHD was almost 4 times, and statistically significant. Gender and blood group mismatch were not found to be significant risk factors. If the relationship of the donor was a parent rather

than a sibling, or the donor was unrelated, the risk was higher, but not statistically significant. For related or unrelated transplants, if the HLA type was not identical, the risk was higher, but not significant. Myeloablative versus reduced intensity conditioning regimen was found to be not significant. Most of the patients received Cyclosporine and Methotrexate combination, therefore the analysis did not reach statistical significance.

Table 25. Risk factors for chronic GVHD:

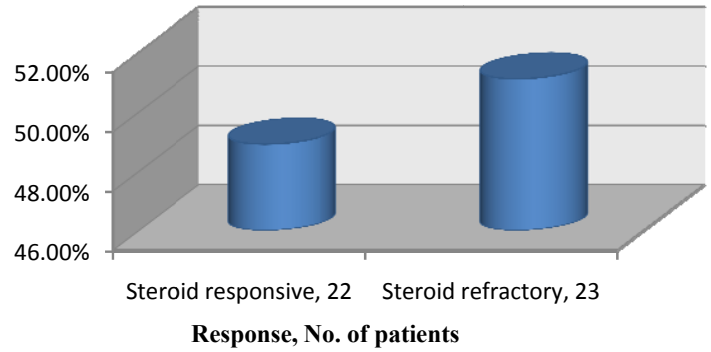
Variable	Risk	95% Confidence intervals	p-value
Patient age <15 years	1		
Patient age ≥15 years	2.191	0.660- 7.270	0.200
Donor age <15 years	1		
Donor age ≥15 years	5.192	1.056- 25.541	0.043
Source of hematopoietic stem cells: Bone marrow	1		
Peripheral blood	6.932	0.830- 57.891	0.074
Gender matched or male donor to female recipient	1		
Female donor to male recipient	1.681	0.466- 6.060	0.427
ABO incompatibility	0.974	0.317- 2.987	0.963
Donor relation: sibling	1		
Parents	2.846	0.363- 22.316	0.319
Unrelated	1.138	0.196- 6.600	0.885
Nonmyeloablative conditioning	1		
Myeloablative Conditioning	0.317	0.095- 1.053	0.061
Related, HLA matched	1		
Mismatched	4.625	0.689- 31.046	0.115
Unrelated, HLA matched	1		
Mismatched	0.250	0.007- 8.560	0.442
Prior acute GVHD	2.470	0.694- 8.790	0.163

Increasing donor age was found to be a statistically significant risk factor for chronic GVHD; the risk was about 5 times higher if the donor was more than 15 years old. With increasing patient age or if the source of the stem cells was peripheral blood compared to bone marrow, there was increased risk of chronic GVHD, but it did not reach statistical significance. Gender or blood group mismatch were not found to be statistically significant risk factors. The risk of chronic GVHD if the donor was not a sibling, but a parent, was higher, but did not achieve statistical significance. Unrelated donor transplant was not a statistically significant risk factor for chronic GVHD. Myeloablative conditioning compared to reduced intensity conditioning was not found to be significant. Mismatched HLA type, if unrelated, and prior acute GVHD were associated with increased risk for chronic GVHD, but they were not statistically significant. For related transplants, mismatched HLA type was not found to be a risk factor. GVHD prophylactic regimens could not be analyzed as all the patients had received Cyclosporine and Methotrexate.

Therapy:

GVHD was treated with corticosteroids as 1st line therapy, if they did not respond, or progressed after 7 days of appropriate steroid therapy, they were treated as per the primary physician's discretion and the therapies used included Mycophenolate, Basiliximab and Rituximab. In a single patient, mesenchymal stem cells were tried. Response to therapy of acute GVHD, when clinically significant disease was treated, is summarized in **Figure 14**.

Figure 14: **Therapy**



Course of the patients and mortality:

A total of 28 patients (27.5%) expired. Cause of death was most often infection-related. A single patient expired due to stage IV gut GVHD, he also had stage I cutaneous GVHD. Another patient died of stage IV liver GVHD, he also had gut GVHD. Primary graft failure was seen in 3 patients, all of them expired early. Six patients (5.9%) relapsed, of whom 3 died during the study period. The causes of death are described in **Table 26**.

Table 26. Cause of death:

S. No.	Cause of death	No. of patients
1	Bacterial sepsis	10
2	Pneumonia- Bacterial	3
	Viral	2
	Fungal	2
3	Intracranial bleed	2
4	Diffuse alveolar hemorrhage	1
5	Primary graft failure	3
6	Relapse	3
7	Grade 4 GVHD	2

DISCUSSION

This study was done to determine the incidence of muco-cutaneous GVHD in a tertiary referral centre in south India between March, 2009 and July, 2010.

Demographic profile:

Out of 102 patients, 43.1% were children. The median age was 20.5 years, signifying that older patients were not considered for transplantation as often as children were. Male: female ratio of the recipients was almost 2:1. Patients were mostly of the higher socio-economic background (88.2%) and were based in India.

Patient profile:

The primary diagnosis was most often AML and thalassemia, followed by aplastic anemia and ALL, then myelodysplastic syndrome, CML, biphenotypic leukemia, Fanconi's anemia, Kostmann syndrome, pure red cell aplasia, juvenile myelomonocytic leukemia, paroxysmal nocturnal hemoglobinuria, Wiskott Aldrich syndrome and severe combined immunodeficiency in decreasing order of frequency.

Patients were asked about a prior history of blood transfusions, which almost all of them had.

Donor compatibility profile:

The donors were most often siblings (77.5%), followed by unrelated donors (14.7%), and parents (7.8%). Donors of all the thalassemia patients were relatives. Unrelated donor transplants were mostly done in patients with AML. Identical HLA typing was found in most of the related donors, 92%. ABO blood group incompatibility

was fairly common, 45.1%. Gender mismatch, from a female donor to a male recipient was seen in 33.3% of the transplants, of which parous donors were 38.2%.

Transplantation profile:

PBSCT was the most common type of transplant, and was performed in all the AML, ALL, CML, biphenotypic leukemia and aplastic anemia patients. BMTs continued to be done in most of the thalassemics. Cord blood transplant was performed in only one patient, who was diagnosed to have Wiskott Aldrich syndrome prenatally. He was one of twins, with an older sibling diagnosed to have the same disease.

More than half the patients received myeloablative conditioning regimens, 53.9%, of which Thiotepa, Treosulphan and Fludarabine was the combination used most often, followed by TBI and Cyclophosphamide. The reduced intensity conditioning regimens used most often consisted of Busulphan, Cyclophosphamide and ATG or Fludarabine and Melphalan.

The median time to engraftment of neutrophils was 16 days, (range, 9-57) and time to engraftment of platelets was 17 days (range, 8-61), slightly longer than two weeks, mentioned by Deeg et al.[15]

Follow up period:

The median follow up was 5½ months. The first patient was followed up for almost 1½ years, and the last patient for three months.

Graft versus host disease:

As the primary outcome of the study, the incidence of GVHD was evaluated in 95 patients, as seven patients expired prior to engraftment. The incidence of GVHD was

found to be 58.9%. The incidence of GVHD is similar to that mentioned in Western literature, upto 80% stated by Wenzel et al.[4]

The incidence of muco-cutaneous GVHD was found to be 38.9%. Although the skin, gut and liver were found to be involved in decreasing order of frequency, as mentioned by Goddard et al,[40] of all the patients with GVHD, muco-cutaneous GVHD comprised only 66.1%, which is lower than Western literature,[14] probably due to difficulty in identifying the faint rashes of acute GVHD, especially after most of the conditioning regimens cause a generalized hyperpigmentation.

Hyperacute GVHD:

Hyperacute GVHD was seen in only one patient, incidence being lower than that mentioned by Saliba et al.[51] It was seen on day 11, slightly later than the usual presentation within the first week.[5] She had an unrelated donor and received peripheral blood stem cells. She had only cutaneous involvement with a maculopapular rash on the body; there was no hepatitis or features of vascular leakage as mentioned by Goker et al.[5] Response to therapy wasn't prompt as opposed to an engraftment syndrome.[15]

Acute GVHD:

Incidence of acute GVHD was 50.9%, within the range 6-90% given by Aractingi et al and Fimiani et al. [38,42]

Acute muco-cutaneous GVHD was seen in 26.3% patients. Most patients had mild clinical stage 1 acute cutaneous GVHD (45.8%). Stage 4 was seen in only one patient, who had a matched unrelated donor transplant and received peripheral blood stem cells. She had a maculopapular rash on day 18 and progressed to have necrolysis in two days; this interval was really short compared to 19 days stated by Goiriz et al.[18] She didn't have

any mucosal involvement, again dissimilar to most of the patients described by Goiriz et al.[18] She was refractory to steroid therapy and subsequently treated with Basiliximab.

Clinical presentation of acute GVHD was most commonly a maculopapular rash, in 14 patients, of which only one progressed to have the appearance of necrolysis of the skin in the form of erosions, as described above. Flexural edematous plaques were seen in a single patient, similar to what is described as eczematoid chronic GVHD,[77] which was grade 3 on biopsy. Follicular GVHD was seen clinically in one patient, who had biopsy features of grade 2 acute GVHD, similar to that described by Tani et al.[57] However, a patient with a maculopapular rash had biopsy features suggestive of follicular GVHD, suggesting that the follicular epithelium was targeted early, as suggested by Friedman et al.[56] Dyspigmentation over the arms, "leopard- like pigmentation" usually seen with chronic GVHD,[9] was seen in one patient on day 60; however, the biopsy was consistent with grade 2 acute GVHD. Mucosal involvement was seen more commonly in the oral mucosa than the glans penis. Twelve patients had white reticulate plaques on the oral mucosa, and not erosions as described by Goiriz et al.[18] One patient had erythema and erosions of the glans, being the sole manifestation of muco-cutaneous GVHD; and another had violaceous plaques on the glans. Oral mucosal involvement with only lips being involved as a cutaneous manifestation, seen as violaceous plaques, was seen in 6 patients. In the patients with acute GVHD, 13 patients had only skin without mucosal involvement, one had only mucosal involvement of the glans penis and 12 had mucosal and skin involvement simultaneously. One patient had a maculopapular rash on day 18, clinical stage 2 and biopsy grade 3, which resolved; and thereafter he had violaceous plaques on the lips and reticulate white plaques on oral mucosa on day 78, clinical stage 2 and biopsy grade 3.

Median time to onset of acute cutaneous GVHD was 32 days, comparable with 30-35 days, mentioned by Hausermann et al and Deeg et al,[3,15] but longer than 19 days mentioned by Schubert et al.[52]

Chronic GVHD:

Chronic GVHD occurred in 27.9% patients, consistent with older literature, at the times when most patients succumbed to acute GVHD.[41,45] But the latest literature says that chronic GVHD is on the rise, upto 80%,[7,8,46] especially in unrelated donor transplants.[34,49] If the patients were followed up longer, the percentage would probably have been higher. Median follow up of patients was 3.2 years (11 months to 5.4 years) in the study done by Fujii et al.[47]

Chronic muco-cutaneous GVHD was seen in 24.6%. It was seen as violaceous plaques on the lips in three of the patients, who also had white reticulate plaques on oral mucosa. Violaceous plaques on the face or rest of the body were seen in three patients. Oral mucosal involvement was in the form of white reticulate plaques, seen in 10 patients (66.7%), slightly lower than 80% incidence mentioned in established data.[42] Only oral mucosal involvement was seen in three patients, without cutaneous manifestations. Two patients had white reticulate plaques on the glans penis. Nail involvement was seen in a single patient with chronic GVHD as splitting, ridging and discoloration, biopsy of the nail was not done to confirm the diagnosis; the patient had concomitant mucosal GVHD, proven histopathologically. Incidence of nail involvement was much lower than 50% mentioned during the Turkish study.[79] Reticulate hyperpigmented plaques with central atrophy and depigmentation on the palms and soles were seen in one patient. Lichen planus-like GVHD was seen in 80% of our patients with chronic GVHD, much higher than 9% mentioned by Schaffer,[9] probably because of the short period of follow up in our

study. Vitiliginous GVHD with depigmented macules on the lips and palms was seen in a patient as late as 497 days post transplant, Fimiani et al described vitiligo like lesions[38] and Williams et al described leukoderma as GVHD.[71] Maculopapular rash on the body without mucosal involvement was seen in two patients, similar to that described by Hymes et al;[65] both of these had prior acute GVHD and developed the rash as immunosuppressive therapy for treatment of the acute GVHD was tapered.

Median time to onset of chronic GVHD was 231 days (range, 130 to 497), longer than 4 months mentioned by Nghiem et al,[69] but within the range 3 to 24 months given by Lee et al.[8]

Quiescent chronic GVHD was seen in 70.6%, comprising the most common type in our setting. It was double that described by Aractingi et al.[42] Progressive chronic GVHD was only found in 5.8%, much less than what was described, whereas the de novo type was 23.5%, similar to established data published by Aractingi et al.[42]

Histopathology:

The 31 patients whose histopathology was consistent with GVHD were treated as GVHD prior to the reporting of the biopsy. In three patients, whose biopsy was not consistent with GVHD, they were not treated as GVHD because of early presentation of the rash, prior to engraftment or the rash was not clinically typical of GVHD. Five patients had two sites biopsied simultaneously to rule out GVHD, three patients had GVHD in both the specimens, but two had GVHD in only one specimen, therefore, we suggest that performing a biopsy from two sites increases the yield of diagnosis. In a single patient, serial biopsies had been done at 15 and 34 days, when he had clinical stages 3 and 2 respectively, but a biopsy revealed grade 1 and 3, indicating that histopathological features take time to be established, as explained by Kuykendall et al.[89]

However, as described by Firoz et al,[58] as the prevalence of GVHD is now described to be >30%, a skin biopsy need not be done to confirm acute GVHD, especially in the first month after the transplant.

Clinico-pathological correlation in patients with acute cutaneous GVHD revealed a negative correlation between clinical stage and histopathological grade, consistent with previously established data.[30]

Risk factors for GVHD:

We also tried to find the relevance of previously known risk factors for acute and chronic GVHD in our population. However, as the patients had differing diagnoses and the sample size was small; many factors did not reach statistical significance.

Acute GVHD:

Increasing patient age and donor age were found to be statistically significant risk factors for acute GVHD; the risk was about 5 times higher if the age was more than 15 years. PBSCT compared to BMT, the risk of acute GVHD was almost 4 times, and statistically significant. PBSCT is said to increase the risk of chronic not acute GVHD.[5] If the relationship of the donor was a parent rather than a sibling, or the donor was unrelated, the risk was higher, but not statistically significant. For related or unrelated transplants, if the HLA type was not identical, the risk was higher, but not significant. HLA mismatch is considered the main risk factor.[15] Gender and blood group mismatch or myeloablative conditioning were found to be insignificant, unlike in other studies.[35,36]

Chronic GVHD:

Increasing donor age was found to be a statistically significant risk factor for chronic GVHD; the risk being 5 times higher if the donor was over 15 years old. With increasing patient age or PBSCT, there was increased risk of chronic GVHD, but it did not reach statistical significance. The risk of chronic GVHD if the donor was not a sibling, but a parent, was higher, but did not achieve statistical significance. Unrelated donor transplant was not a statistically significant risk factor for chronic GVHD. Gender or blood group mismatch and myeloablative conditioning were not found to be statistically significant risk factors. Prior acute GVHD was associated with increased risk for chronic GVHD, but was not statistically significant. The main risk factor is thought to be prior acute GVHD,[8,38] however, in the study in Japan, they found no difference in the incidence of chronic GVHD whether the patient had prior acute GVHD or not.[47] Mismatched HLA type was not found to be a statistically significant risk factor, whether related or not.

Therapy:

Around half the patients were responsive to steroid therapy; the others were treated with Mycophenolate, Basiliximab or Rituximab. Mesenchymal stem cells were used in a single patient, but he succumbed to the disease.

Course of the patients:

Many patients, 27.5% expired, mostly due to infectious causes. Two patients died of GVHD. Three patients had primary graft failure and another three relapsed.

CONCLUSION

- The incidence of acute muco-cutaneous GVHD was 26.3%, the most common presentation being a maculopapular rash. Median time to onset was 32 days (range, 11 to 95).
- The incidence of chronic muco-cutaneous GVHD was only 24.6%, probably due to short follow up period. Median time to onset was 231 days (130-497).
- Incidence of lichenoid chronic GVHD was 80%.
- Maculopapular rash occurred in 2 patients as part of chronic GVHD, both were refractory to steroid therapy.
- Quiescent chronic GVHD is the commonest type of chronic muco-cutaneous GVHD in India.
- Clinical and histopathological grades of acute cutaneous GVHD didn't show correlation in our study.
- Among the risk factors, older patient age, donor age and PBSCT were found to be significant for acute GVHD and older donor age for chronic GVHD.
- Mortality rate was 27.5%.
- The incidence of GVHD was found to be 58.9% in India, muco-cutaneous GVHD was 38.9%, gut GVHD was 36.8% and liver GVHD was 32.6%.

LIMITATIONS

1. Rashes of acute graft versus host disease are often very faint and difficult to recognize on darker skin, especially as the conditioning regimen causes most of the patients to become hyperpigmented.

2. Judgment of the presence of acral erythema is difficult.

3. Once the diagnosis of graft versus host disease was established, skin biopsy was not repeated, when the patient progressed to a worse clinical grade in a few days.

4. Acute and chronic GVHD was classified by the traditional 100 day criteria as the definitions for overlap in muco-cutaneous GVHD are not well defined.

5. Evaluation of sclerodermatous GVHD was not possible due to the short duration of the study.

RECOMMENDATIONS

- All hematopoietic stem cell transplant patients should be regularly followed up by a dermatologist.
- Any rash occurring prior to engraftment should be considered for biopsy to rule out hyperacute GVHD.
- A new staging system is required for acute GVHD which includes purely mucosal involvement.
- Overlap syndrome should be more clearly defined in patients with muco-cutaneous GVHD.
- Patients need to be followed up for many years to be able to assess sclerodermoid spectrum of GVHD.
- Routinely performing a skin biopsy in the first month in case a rash develops is not indicated in our population as the incidence of graft versus host disease is 38.9% and many patients develop GVHD in other organs as well. However, histopathological diagnosis is important to rule out another diagnosis where considered, especially infections.
- Biopsy from two sites may be more helpful since the yield is better.
- Histopathological grading of acute cutaneous GVHD does not correlate with clinical staging and so may not be helpful.

SUMMARY

Background:

Allogeneic hematopoietic stem cell transplants are being increasingly performed for various indications. GVHD is the most common complication of hematopoietic stem cell transplantation. Skin is the organ affected most often by this process.

Objective:

To determine the incidence of muco-cutaneous GVHD, describe the clinical presentations, establish the clinico-pathological correlation of acute cutaneous GVHD and analyze the risk factors for GVHD in India.

Methodology:

A single centre prospective longitudinal study was performed in Christian Medical College, Vellore with 102 patients from March, 2009 to July, 2010. All patients undergoing hematopoietic stem cell transplantation were screened and recruited into the study prior to the transplant, after obtaining a written informed consent and an assent form for children where applicable, which was approved by the institutional review board. All patients were examined by the principal investigator prior to the transplant and periodically thereafter, every week or month, as the patient followed up, or if any muco-cutaneous symptom appeared. The presence and extent of skin rash and oral mucosal involvement was clinically assessed, photographs were taken and biopsies performed where indicated, to help confirm the diagnosis, with the patient's consent. Routine investigations, including total count, differential count and liver function tests were done regularly. Demographic data was collected from the records, as was the pre-transplant diagnosis, donor related information, stem cell source and conditioning regimen. The

calculated sample size was 92. All statistical analysis was performed using SPSS 11.0 for windows (SPSS Inc, Chicago, IL). Frequency and percentages were used to describe the distribution of categorical variables, median and ranges were used to describe continuous variable that were not normal distributed. Chi-square test was used to assess the association between categorical variables. Continuous variables were compared using t-test. Weighted kappa statistic was used to assess agreement between clinical and histopathological grades of GVHD; squared weights were employed. The agreement was analyzed using R 2.8.0 ("irr" library). Logistic regression was employed to estimate the adjusted odds ratios as measure of risk for GVHD against potential risk factors. A p-value of less than 0.05 was considered statistically significant.

Results:

Out of a sample size of 102 patients, 95 were evaluated for acute GVHD and 61 for chronic GVHD. Median age was 20.5 years. Males comprised 65.7% of the patient population. The most common primary diagnosis was AML, followed by thalassemia. Donors were mostly siblings, 77.4%; and unrelated donors comprised 14.7%. HLA type was identical in 92% of related transplants. ABO blood group incompatibility was seen in 45.1%. Gender mismatch from a female donor to male recipient was 33.3%. Peripheral blood was the most common source of hematopoietic stem cells, in 69.6%. Myeloablative conditioning was given for 53.9% patients. Cyclosporine and Methotrexate combination was given as prophylaxis for 94.1% patients. The median time to engraftment of neutrophils was 16 days (range, 9 to 57) and platelets was 17 days (range, 8 to 61). Acute muco-cutaneous GVHD was seen in 26.3% and chronic in 24.6% patients. Median time to onset of acute GVHD was 32 days (range, 11 to 95) and chronic GVHD was 231 (range, 130 to 497). Incidence of GVHD was found to be 58.9% in our population. There

was a negative clinico-pathological correlation in acute cutaneous GVHD. Most of the risk factors analyzed did not reach statistical significance due to the presence of a heterogeneous population with differing diagnoses. However, older patient and donor age, and PBSCT were found to be significant risk factors for acute GVHD and older donor age for chronic GVHD.

Conclusion:

The incidence of acute muco-cutaneous GVHD was 26.3%, most commonly a maculopapular rash occuring around 32 days (range, 11 to 95). The incidence of chronic muco-cutaneous GVHD was 24.6%, most commonly lichenoid GVHD occurring around day 231 (130-497). Quiescent chronic GVHD is the most common type.

Clinical and biopsy grades had no correlation in acute cutaneous GVHD. Older patient and donor age and PBSCT were risk factors for acute GVHD and older donor age was a significant risk factor for chronic GVHD.

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ANNEXURES

Annexure 1

Subject Information Sheet / Informed consent Form to Participate in a Research Study

Study title: "Study of the incidence of muco-cutaneous Graft versus Host Disease among patients undergoing allogenic hematopoeitic stem cell transplantation"

Principal investigator: Dr. Anisha Chandy, Department of Dermatology, CMC, Vellore

Institution: Christian Medical College, Vellore (Departments of Dermatology and Haematology)

Introduction: This study is being done to find out the number of patients that have a reaction to the bone marrow transplant in the skin or mucosa. It requires that you should be regularly followed up to watch for any muco-cutaneous signs.

Study procedures: You will be examined by doctors in the Haematology department and will be asked to undergo tests which are done as part of routine protocol for any patient undergoing hematopoeitic stem cell transplantation. In case a clinical diagnosis is difficult, a biopsy from the skin or mucosa may need to be done. A photograph, if necessary, will be taken with your permission. No additional tests will be done.

Benefits/risks: None.

Confidentiality/Privacy: Strict privacy will be maintained during the interview, clinical examination and information of laboratory results. Your name will not appear on the study records, but will be linked to them by a study number which will be kept confidential by the study investigator.

Contacts: If you experience any unforeseen difficulty from the study, you may come back to the Haematology department or the Emergency department immediately or contact Dr. Anisha Chandy at 2283403.

If you have any queries regarding the Ethical aspects of the study you can contact the below address:

Ethics Committee address/ Chairperson contact number:

Dr. L. Jeyaseelan Ph.D.

Secretary, Institutional Review Board,

(Ethics Committee)

Christian Medical College,

Vellore- 632 002, Tamil Nadu, India

91 416 2284205 / 2262703

Consent by the Subject:

Signature of the subject:

Date:

Name:

Hospital No.:

Serial No:

Signature of impartial witness (if applicable)/ signature of the LAR (if illiterate):

Relationship to the patient:

Signature:

Name:

Date:

Person conducting ICF/ designate (PI/Co-PI):

Signature:

Date:

Name:

Annexure 2

Departments of Dermatology & Haematology,

Christian Medical College,

Vellore- 632004

Assent Form for Children

Title of Study:

Study of the incidence of muco-cutaneous Graft versus Host Disease among patients undergoing allogeneic hematopoietic stem cell transplantation"

Principal Investigators:

Dr. Anisha Chandy, Department of Dermatology, CMC, Vellore

Why are we doing this study?

This study is being done to find out the number of patients that have a reaction to the bone marrow transplant in the skin or mucosa. It requires that you should be regularly followed up to watch for any muco-cutaneous signs.

What will happen during the study?

You will be examined by doctors in the Haematology department and will be asked to undergo tests which are done as part of routine protocol for any patient undergoing hematopoietic stem cell transplantation. In case a clinical diagnosis is difficult, a biopsy from the skin or mucosa may need to be done. A photograph, if necessary, will be taken with your permission. No additional tests will be done.

Are there good things and bad things about the study?

No.

Who will know about what I did in the study?

If you are a part of this study, your name and address will not be given to anyone without your consent.

Can I decide if I want to be in the study?

Nobody will be angry or upset with you if you do not want to be in this study. We are talking to your parents/ legal guardians about the study and you should talk to them about it too.

Assent:

I was present when_____ read this form and gave my verbal assent.

Name of the Patient	Signature	Date

Name of the person who obtained consent	Signature	Date

Annexure 3

PROFORMA

Serial No.

Name Hospital No. Age

Address

Sex

Occupation

Marital status

Religion

Education(highest class studied)

Locality-urban

-rural

Monthly income:

Diagnosis:

Duration of illness:

Type of transplant:

Date of transplant:

Source of haematopoietic stem cells:

HLA type:

Blood group:

History of prior drug allergies:

History of prior blood transfusions

Name of donor:

Age:

Sex:

Hospital No:

Relation:

HLA type:

Blood group:

History of pregnancy:

History of prior blood transfusions

Date	Extent of Rash	Distribution	Morphology	Mucosal involvement	Hair involvement	Nail involvement	Icterus	Diarrhoea

Pre-transplant checkup:

Date of examination:

Conditioning regimen:

GVHD prophylaxis:

Clinical findings:

Post-transplant checkup:
Acute GVHD:
Grade:
Overlap syndrome:
Chronic GVHD:
Lichenoid:
Sclerodermoid:
Limited:
Extensive:

Lab parameters

Date	Total Count	Differential Count	Bilirubin	Biopsy No.	Histopathological grade/ findings

Clinico-pathological co-relation:

Annexure 4

CHRISTIAN MEDICAL COLLEGE
VELLORE - 632 002, INDIA.
INSTITUTIONAL REVIEW BOARD (IRB)

Dr. George Thomas, D.Orth
Editor Indian Journal of Medical Ethics
Chairman, Ethics Committee

Dr. George Mathew, MS,MD,FCAMS
Chairman, Research Committee &
Principal

Dr. Shuba Kumar, PhD
Deputy Chairman, Ethics Committee

Dr. Gagandeep Kang, MD,PhD,FRCPath
Deputy Chairman, IRB &
Additional Vice Principal (Research)

Dr. L. Jeyaseelan, MSc,PhD
Secretary, IRB

March 24, 2009

Dr. Anisha Chandy
PG Registrar
Department of Dermatology
Christian Medical College
Vellore 632 004

Sub: FLUID Research grant project NEW PROPOSAL:
Study of the incidence of muco-cutaneous Graft versus Host Disease among patients undergoing allogenic hematopoeitic stem cell transplantation in an Indian setting.
Dr. Anisha Chandy, PG Registrar, Dermatology, Dr. Susanne A Pulimood, Dermatology, Dr. Alok Srivastava, Haematology, Dr. Laxmisha Chandrashekar, Dr. Dincy Peter, Dermatology, Dr. Mammen Chandy, Dr. Vikram Mathews, Dr. Auro Viswabandya, Haematology, Dr. Meera Thomas, General Pathology.

Ref: IRB Min. No. 6787 dated 18.03.2009

Dear Dr.Anisha Chandy,

The Institutional Review Board (Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Study of the incidence of muco-cutaneous Graft versus Host Disease among patients undergoing allogenic hematopoeitic stem cell transplantation in an Indian setting" on March 18, 2009. I am quoting below the minutes of the meeting.

The Committees reviewed the following documents:

1. Format for application to IRB submission.
2. Study Proforma (English).
3. Informed Consent Form (English, Tamil, Hindi, Bengali).
4. Present knowledge and relevant bibliography.
5. A CD containing documents 1-4.

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788
e-mail : research@cmcvellore.ac.in

CHRISTIAN MEDICAL COLLEGE
VELLORE - 632 002, INDIA.
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Dr. Shuba Kumar, PhD
Deputy Chairman, Ethics Committee

Dr. Gagandeep Kang, MD,PhD,FRCPath
Deputy Chairman, IRB &
Additional Vice Principal (Research)

Dr. L. Jeyaseelan, MSc,PhD
Secretary, IRB

The following Ethics Committee members were present at the meeting held on March 18, 2009 at 10:00 am in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Other Affiliations
Dr. George Thomas	MBBS, D.Ortho	Chairperson (IRB) & Orthopaedic Surgeon, St. Isabel Hospital, Chennai & Editor, Indian Journal of Medical Ethics	Non-CMC Staff.
Dr. Shuba Kumar	MA, MSc, Ph.D.	Dy. Chairperson (IRB) & Social Scientist, SAMRATH, Chennai.	Non-CMC Staff.
Dr. L. Jeyaseelan	MSc, PhD, FRSS	Professor & Head Dept. of Biostatistics & Secretary IRB (EC), CMC	
Dr. George Mathew	MBBS, MS, MD	Principal, C.M.C.	
Dr. Thambu David (on behalf of Dr. Lionel Gnanaraj)	MBBS, MS, M.Ch. (Urol)	Medical Superintendent, CMC.	
Dr. Prathap Tharyan	MD, MRCPsych.	Associate Director, Professor of Psychiatry, CMC	
Mrs. Shirley David (on behalf of Mrs. Bharathy Jacob)	M.Sc. (Nursing), RN, RM	Dean, College of Nursing, CMC.	
Rev. Malhia Joshua (on behalf of Rev. Dr. T. Arul Dhas)	M.Sc., BD, Ph.D.	Chaplain, CMC	
Mr. Harikrishnan	BL.	Lawyer	Non-CMC staff.
Dr. Denny Fleming	MBBS, MD	Professor, Pharmacology Dept. CMC.	
Mrs. Radha Anil	M.Sc.	Correspondent, Apple Kids, Sathuvachari, Vellore.	Non-CMC staff.
Rev. Dr.S.G.Immanuel	PhD, MDIV	Pastor, Vellore	Non-CMC-Staff
Mrs. S. Pattabhiraman	BSc, DSSA	Social Worker, Vellore	Non-CMC-Staff
Dr. Gagandeep Kang	MD, PhD, FRCPath.	Dy. Chairperson (IRB), Professor of Microbiology & Addl. Vice Principal (Research), CMC.	

A sum of Rs. 59,500/- (Rupees fifty nine thousand five hundred only) is sanctioned for 2 years out of which a maximum of Rs. 1,500/- can be spent for stationery, printing, Xeroxing and computer charges (if computers used are within the institution).

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788
e-mail : research@cmcvellore.ac.in

CHRISTIAN MEDICAL COLLEGE
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Chairman, Research Committee &
Principal

Dr. Gagandeep Kang, MD,PhD,FRCPath
Deputy Chairman, IRB &
Additional Vice Principal (Research)

The Institutional Ethics Committee / Independent Ethics Committee expects to be informed about the progress of the project, any SAE occurring in the course of the project, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

Yours sincerely,

L. Jeya...

Dr. L. Jeyaseelan Ph.D.
Secretary, Institutional Review Board

Secretary
Institutional Review Board
(Ethics Committee)
Christian Medical College
Vellore - 632 002, Tamil Nadu, India

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788
e-mail : research@cmcvellore.ac.in

GLOSSARY TO THE MASTER TABLE

Sex:

1. Male
2. Female

Mstatus: Marital status

0. Not applicable
1. Married
2. Single
3. Widow

Locat: Locality

1. Urban
2. Rural

Diag: Diagnosis:

1. Thalassemia
2. Acute myeloid leukemia (AML)
3. Acute lymphoblastic leukemia (ALL)
4. Aplastic anaemia
5. Myelodysplastic syndrome
6. Biphenotypic leukemia
7. Kostmann syndrome
8. Fanconi's anaemia
9. Pure red cell aplasia
10. Juvenile myelomonocytic leukemia
11. Paroxysmal nocturnal hemoglobinuria
12. Chronic myeloid leukemia (CML)
13. Wiskott Aldrich Syndrome
14. Severe combined immunodeficiency

Sour: Source of haematopoeitic stem cells:

1. Bone marrow
2. Peripheral blood
3. Cord blood

HLA type:

1. Identical
2. 9/10
3. Haplomatch
4. 5/6
5. 7/8
6. 8/10
7. 4/6

Bld grp: Blood group:

1. O+
2. B+
3. AB+
4. A+

5. A-
6. O-
7. B-
8. AB-

Drug aller: History of prior drug allergies:

0. No
1. Yes

Bld trans: History of prior blood transfusions:

0. No
1. Yes

Relat: Donor relation:

0. Unrelated
1. Sister
2. Brother
3. Father
4. Mother

Preg: History of pregnancy:

0. No
1. Yes

Cond: Conditioning regimen:

1. Fludarabine and Melphalan
2. Busulphan, Cyclophosphamide and Anti-thymocyte globulin (ATG)
3. Total body irradiation (TBI), Cyclophosphamide and ATG
4. Etoposide, TBI, Cyclophosphamide and ATG
5. Idarubicin, Cytarabine, Fludarabine and Melphalan (FLAG-IDA)
6. Fludarabine and Cyclophosphamide
7. TBI and Cyclophosphamide
8. Busulphan and Cyclophosphamide
9. Idarubicin, Cytarabine and Fludarabine
10. Fludarabine, Cyclophosphamide and ATG
11. Fludarabine and TBI
12. Busulphan, Fludarabine and Melphalan
13. Thiotepa, Treosulphan and Fludarabine
14. Busulphan, Cyclophosphamide and Melphalan
15. Busulphan, Etoposide and Cyclophosphamide
16. Fludarabine and Busulphan
17. Cyclophosphamide and ATG
18. Treosulphan and Fludarabine

Px: GVHD prophylaxis:

1. Cyclosporine (CsA) and Methotrexate
2. Cyclosporine
3. Methotrexate
4. Methotrexate and Tacrolimus
5. CsA & Mycophenolate moefetil (MMF)

Rx: Treatment:

0. No
1. Methyl Prednisolone
2. Mycophenolate
3. Methyl prednisolone and Mycophenolate
4. Methyl prednisolone, Mycophenolate, Basiliximab
5. Methylprednisolone and basiliximab

E_ANC: Neutrophil engraftment
E-plts: Platelet engraftment

Ac GVHD: Acute GVHD
Chr GVHD: Chronic GVHD
Liver: Liver GVHD
Gut: Gastrointestinal GVHD

0. No
1. Yes

FU1: First follow up

GVHD:

0. No
1. Mucosal
2. Cutaneous
3. Skin and mucosa

Gd: Clinical grade:

0. <25% body surface area involvement
1. 25-50% body surface area involvement
2. >50% involvement
3. Bullae

Bx Gd: Histopathological grade:

1. Basal vacuolar change
2. With dyskeratotic keratinocytes as well
3. With focal clefting of the basal layer
4. Total separation of the epidermis from dermis

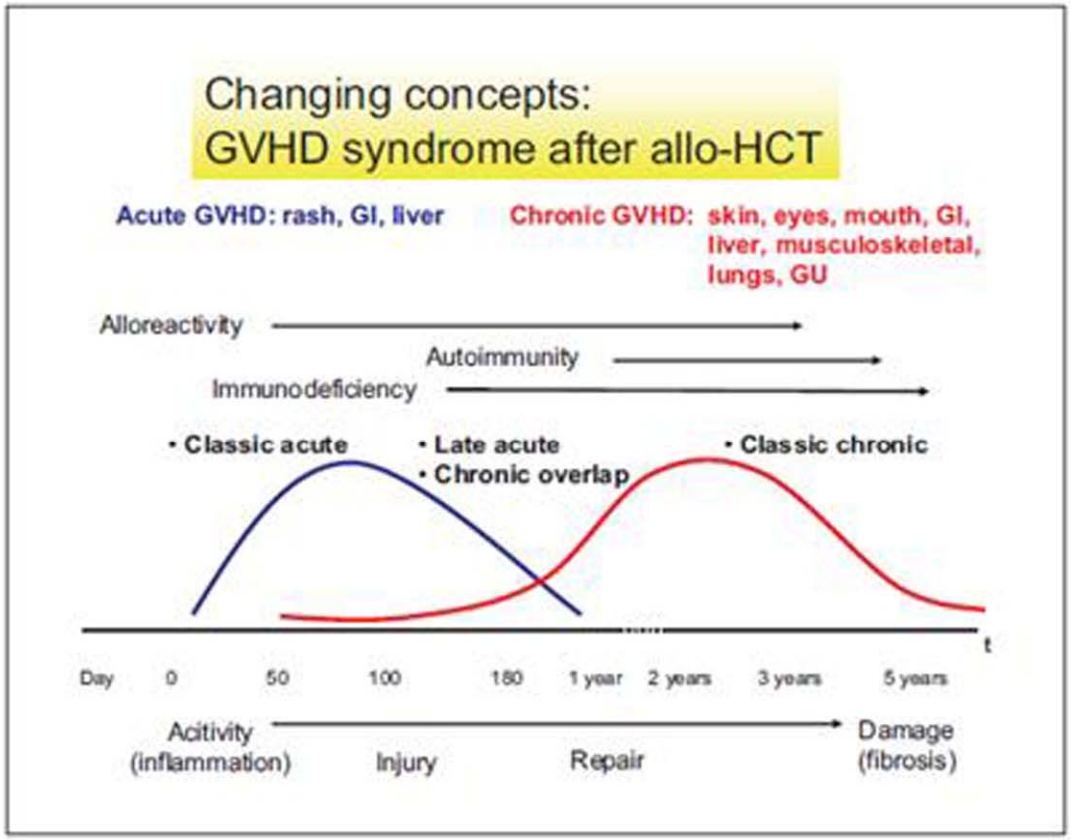
FU2: Second follow up
FU3: Third follow up
FU4: Last follow up

Relap: Relapse

Exp: Expired

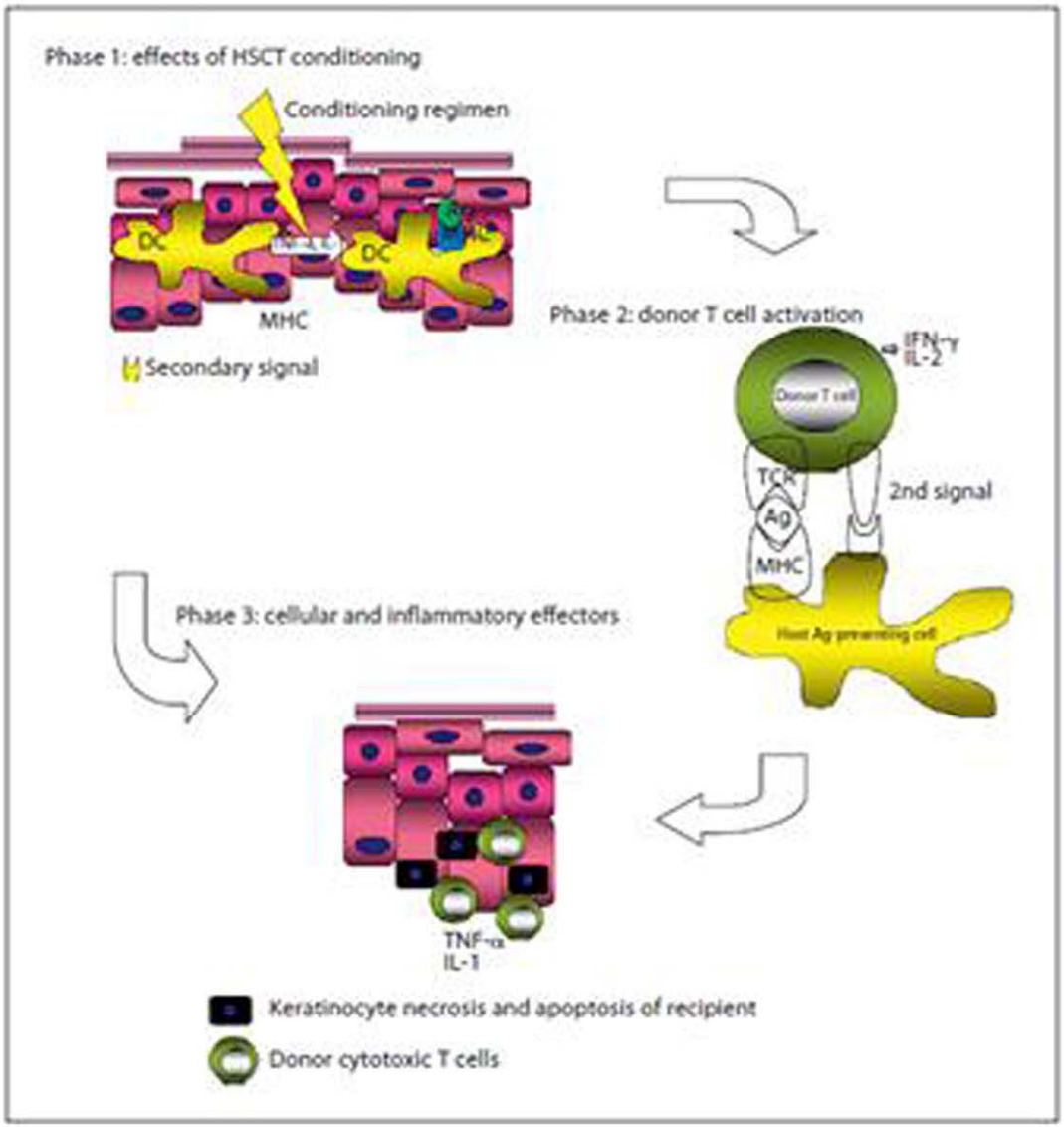
GVHD, figures

Figure 1



GI=gastrointestinal; GU=genito-urinary.

Figure 2



DC=Dendritic cell; Ag=Antigen; TCR=T cell receptor.

Figure 17: Erythema & erosions of the glans penis in acute GVHD

Figure 19: White reticulate plaques on the buccal mucosa

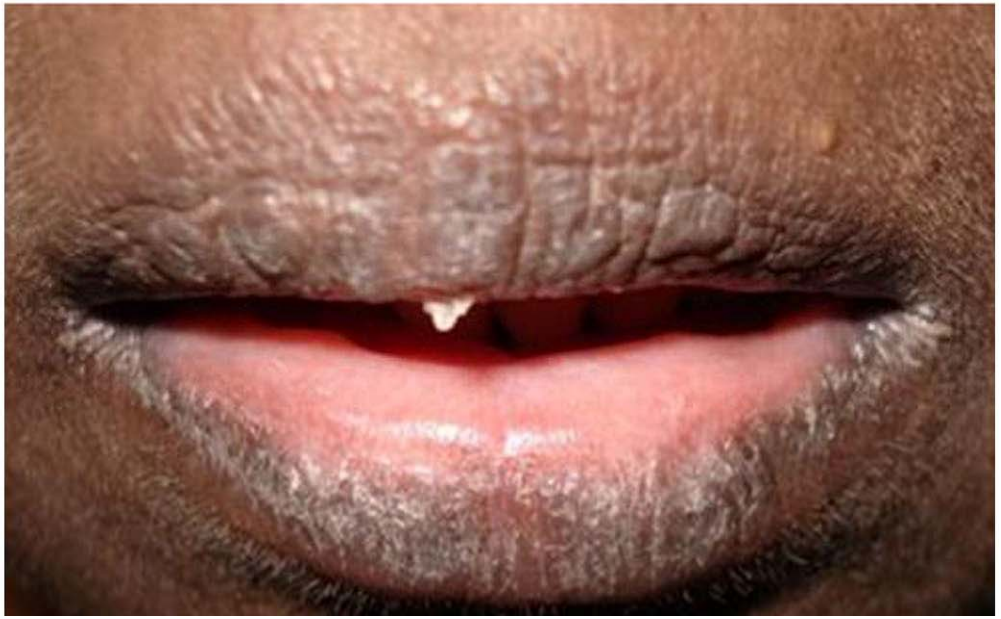
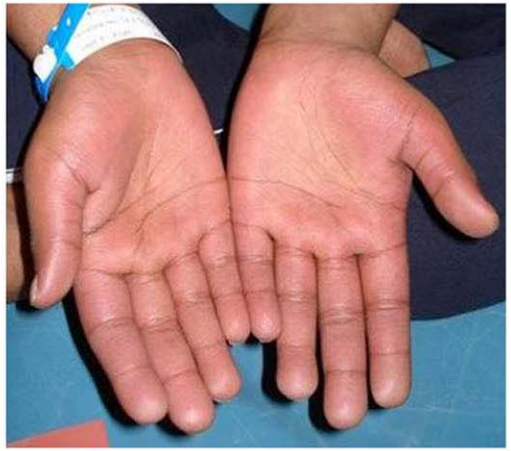


Figure 21: Stage IV acute GVHD

Figure 23: Acute follicular GVHD

Figures 25 & 26: Faint erythematous rash of Stage III acute GVHD



Figures 27 & 28: Recurrence of acute GVHD with acral involvement

Figures 29 & 30: Maculopapular rash in Stage III acute GVHD

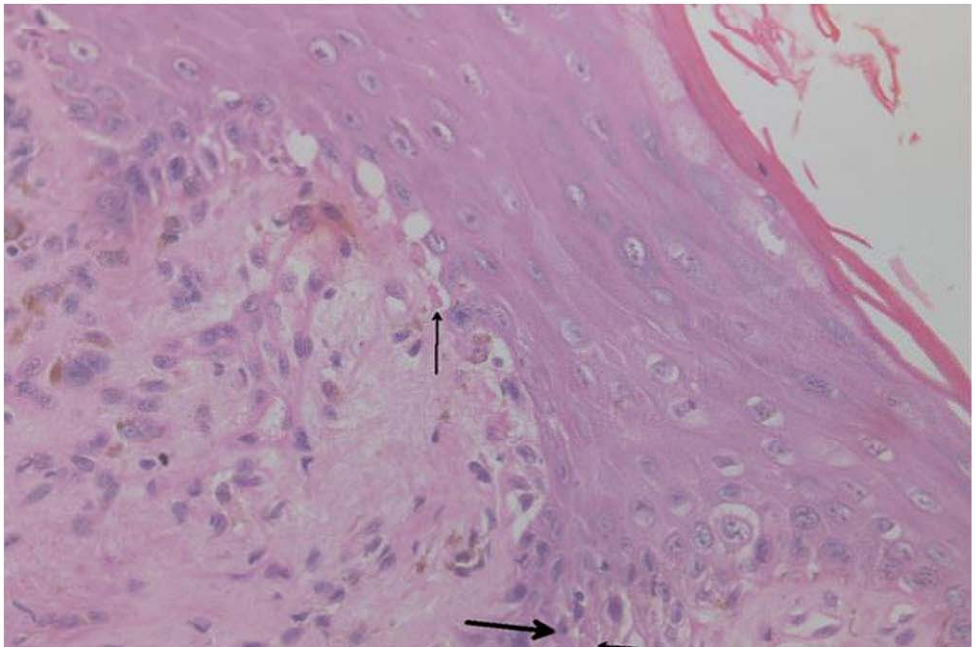
Figure 33: Violaceous discoloration in chronic GVHD

Figure 35: White reticulate plaques on the glans penis in chronic GVHD

Figure 36: Nail involvement in

Figure 37: Violaceous plaques of lichenoid GVHD

Figure 39: Vitiliginous GVHD



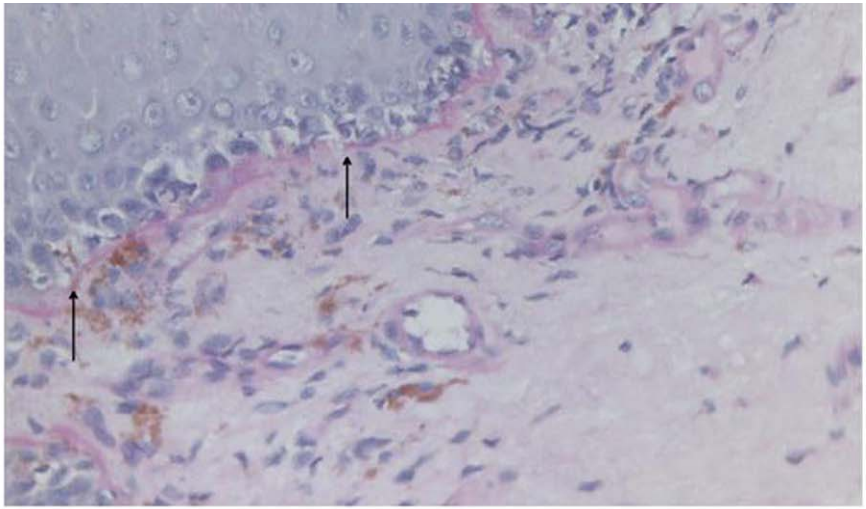
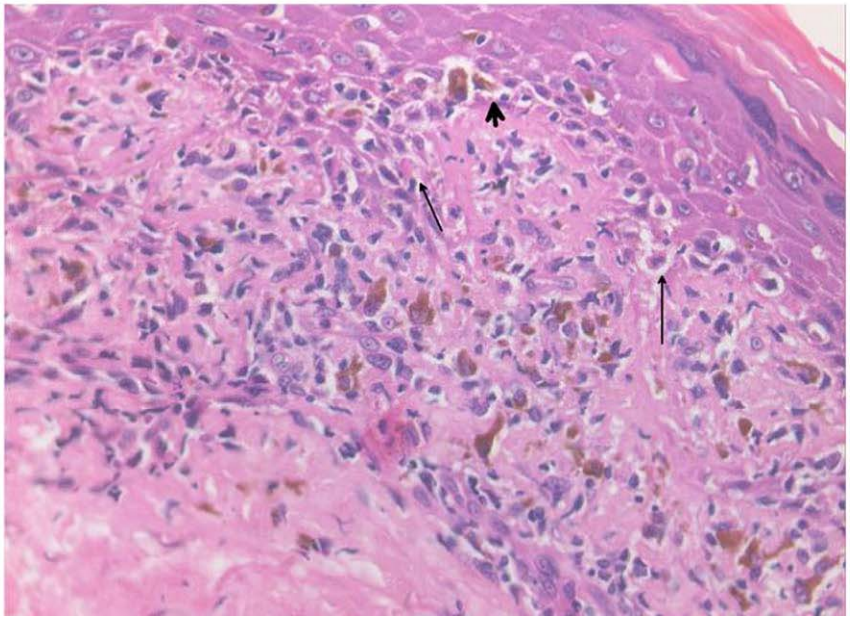


Figure 41: Basal cell vacuolation with lymphocytic exocytosis, PAS, 400X



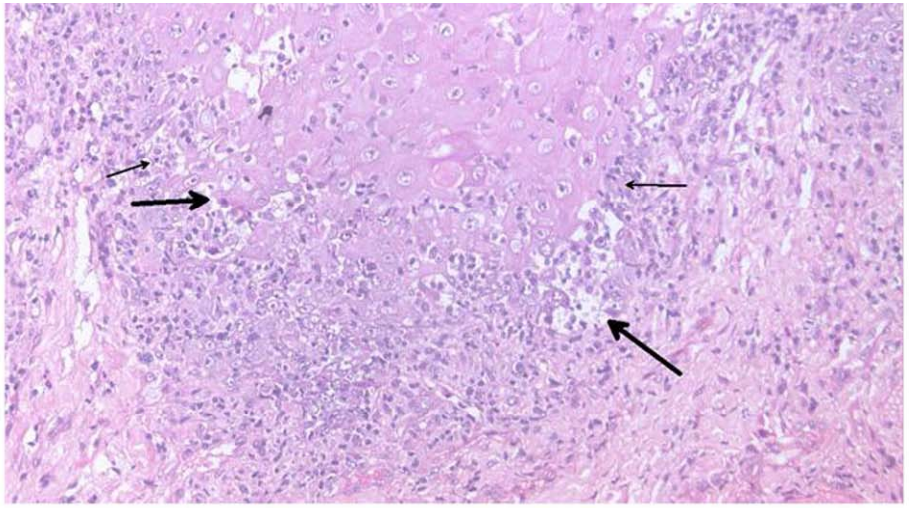
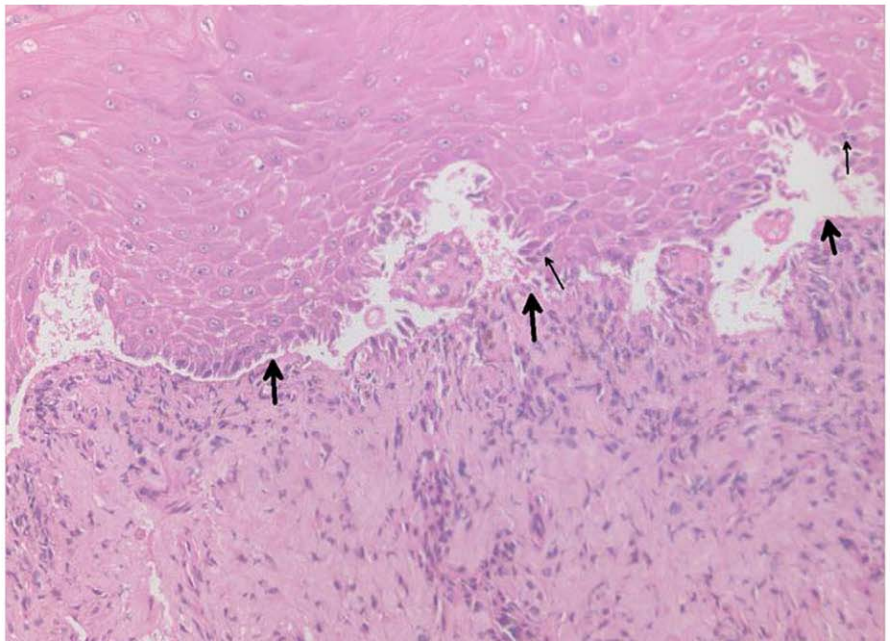


Figure 43: Grade 3 GVHD with focal clefting, H&E, 200X



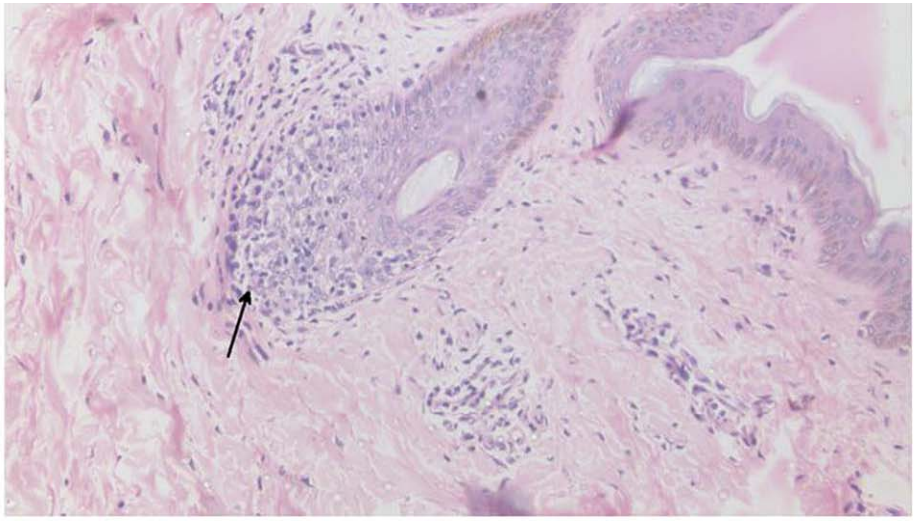
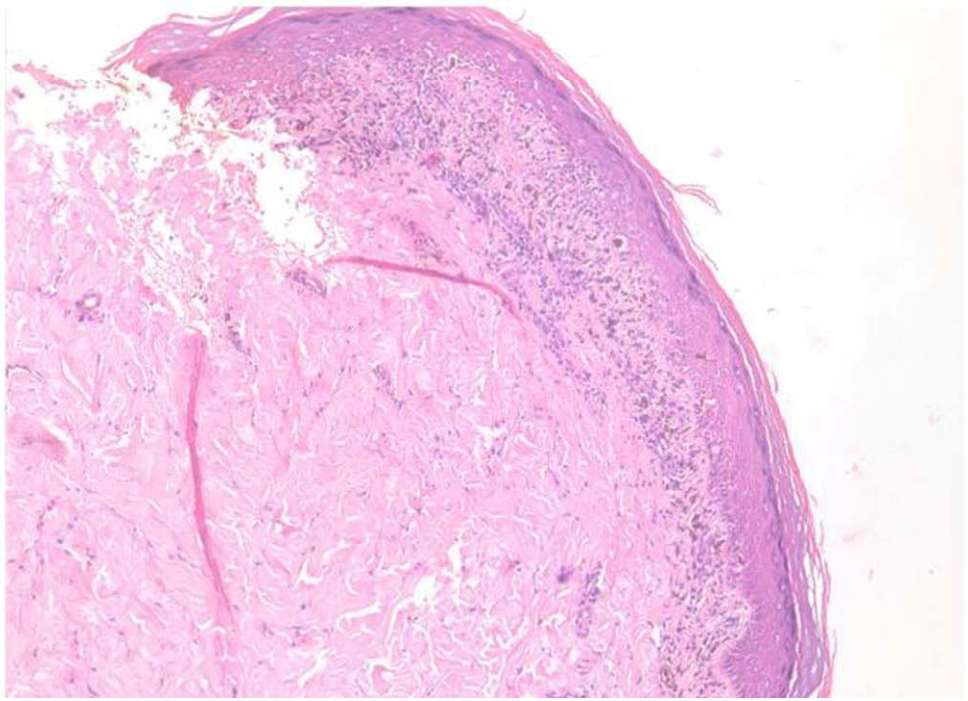


Figure 45: Grade 2 follicular GVHD, H&E, 200X



Sno	Hos.No	Age	Sex	Mstatus	Edu	Locat	Diag	Source	HLA	Bld grp	Drug aller	Bld trans	D_Age	D_Sex	Relat
1	359345D	15	1	0	3	1	2	2	1	1	0	1	26	2	1
2	029021D	9	2	0	1	1	1	1	1	3	0	1	4	2	1
3	359149D	42	2	1	3	2	2	2	4	4	1	1	45	1	2
4	339446D	23	1	2	5	1	2	2	2	1	0	1	45	1	0
5	330935D	21	1	2	6	2	3	2	6	3	0	1	45	1	0
6	387868D	54	1	1	3	1	2	2	1	4	1	1	56	1	2
7	137279D	42	2	1	3	1	4	2	1	4	0	1	38	1	2
8	386854D	58	1	1	4	1	4	2	1	1	0	1	44	1	2
9	301205D	35	1	1	5	1	4	2	1	2	0	1	38	2	1
10	363027D	53	2	1	3	1	5	2	1	2	1	1	58	1	2
11	385496D	32	1	1	6	1	6	2	1	1	0	1	24	1	2
12	275604D	1	1	0	0	1	7	1	1	1	0	1	11	2	1
13	373321D	32	2	1	3	2	4	2	1	1	0	1	35	1	2
14	159107D	7	1	0	1	1	1	1	1	2	0	1	7	2	1
15	424943D	28	1	1	3	1	2	2	1	1	0	1	30	2	1
16	281179D	48	2	1	3	1	4	2	1	1	0	1	40	1	2
17	347234D	26	1	2	6	1	2	2	1	4	0	1	24	2	1
18	408536D	40	1	1	2	1	2	2	1	1	0	1	39	1	2
19	393560D	36	1	1	6	1	2	2	1	1	1	1	39	1	2
20	318634D	11	2	0	2	1	1	1	1	1	0	1	9	2	1
21	380311D	43	1	1	4	1	2	2	5	2	0	1	50	1	0
22	394217D	30	1	1	5	1	2	2	2	2	0	1	22	1	0
23	448962C	49	1	1	4	1	2	2	1	4	1	1	41	1	2
24	792367C	12	1	0	1	1	1	1	1	2	0	1	24	1	2
25	437129D	10	1	0	1	1	2	2	1	4	0	1	17	2	1
26	528515B	48	1	1	5	1	2	2	1	4	0	1	46	1	0
27	665228C	4	1	0	0	2	1	1	1	1	0	1	13	2	1
28	386986D	12	2	0	2	1	4	2	4	1	0	1	22	1	2
29	418816D	38	1	1	6	1	3	2	4	4	0	1	34	2	1
30	430307D	25	2	2	5	1	4	2	2	4	1	1	22	1	2
31	363848D	10	1	0	2	1	8	1	1	2	0	1	3	2	1
32	415095D	22	1	2	5	1	2	2	1	1	1	1	25	1	2
33	441405D	58	2	1	6	1	2	2	1	1	0	1	49	1	2
34	197353D	14	1	0	3	1	1	1	1	2	0	1	10	1	2
35	217525D	9	2	0	1	1	1	1	1	7	0	1	6	2	1
36	486153D	2	2	0	0	1	1	1	1	1	0	1	9	2	1
37	607951C	4	2	0	0	1	9	1	1	1	0	1	11	1	2
38	401517D	11	1	0	2	1	4	2	1	2	0	1	7	1	2

39	467638D	47	1	1	3	2	2	2	1	4	0	1	45	1	2
Sno	**Hos.No**	**Age**	**Sex**	**Mstatus**	**Edu**	**Locat**	**Diag**	**Source**	**HLA**	**Bld grp**	**Drug aller**	**Bld trans**	**D_Age**	**D_Sex**	**Relat**
40	938956C	8	2	0	1	1	1	1	1	1	0	1	3	1	2
41	481690D	34	2	1	6	1	2	2	1	1	0	1	34	1	2
42	443770D	22	2	2	5	1	5	2	1	6	0	1	26	2	1
43	572223C	7	2	0	1	1	1	1	1	2	0	1	42	1	3
44	398586D	9	1	0	1	1	2	2	3	4	0	1	35	2	4
45	638644C	5	1	0	0	1	1	1	1	4	0	1	8	2	1
46	385444D	17	2	0	3	1	1	2	1	4	0	1	55	1	3
47	516034D	3	1	0	0	1	10	2	1	1	0	1	4	2	1
48	381248D	47	1	1	5	1	5	2	2	1	0	1	28	1	0
49	384952D	35	1	1	6	1	2	2	2	3	0	1	33	1	0
50	544858D	28	1	1	6	1	3	2	1	1	0	1	24	1	2
51	371294D	8	2	0	1	1	1	1	4	1	0	1	26	2	4
52	453019D	31	1	1	6	1	6	2	1	2	0	1	33	1	0
53	543672D	3	2	0	0	1	4	2	1	3	1	1	39	1	3
54	325613D	25	1	2	6	1	5	1	2	4	0	1	33	1	0
55	329067D	11	1	0	2	1	1	1	1	6	0	1	4	2	1
56	377904D	54	1	1	3	2	4	2	1	4	0	1	60	1	2
57	465317D	34	1	2	5	1	2	2	1	1	1	1	36	2	1
58	200245D	39	1	1	4	2	3	2	1	3	0	1	34	2	1
59	551137D	14	1	0	3	1	1	1	1	4	0	1	11	2	1
60	611136C	18	1	0	4	2	2	2	2	1	1	1	43	2	0
61	451896D	34	1	1	5	1	2	2	1	1	0	1	42	1	2
62	452232D	23	1	2	5	1	11	2	1	1	0	1	32	1	0
63	046016D	10	1	0	1	1	1	1	1	2	0	1	5	1	2
64	045009D	3	1	0	0	1	1	2	1	2	0	1	7	2	1
65	354423D	4	1	0	0	1	4	2	4	1	0	1	34	1	3
66	297850D	4	2	0	0	1	4	2	1	1	0	1	14	2	1
67	396304D	33	2	1	6	1	2	2	2	2	1	1	33	1	0
68	508501D	32	2	1	2	2	2	2	1	6	0	1	39	1	2
69	764652C	33	1	1	5	1	3	2	1	3	0	1	26	2	1
70	526096D	11	1	0	2	1	2	2	1	1	0	1	42	2	4
71	115870C	12	2	0	2	1	1	1	1	4	0	1	10	1	2
72	575869C	14	2	0	3	1	1	1	1	1	0	1	21	2	1
73	483616D	51	2	1	5	1	12	2	1	1	1	1	56	1	2
74	249086D	9	1	0	1	2	1	1	1	4	0	1	4	2	1
75	452069D	7	1	0	1	1	1	1	1	1	0	1	3	1	2
76	465938D	0.6	1	0	0	1	13	3	1	2	0	0	7	2	1

77	607088D	18	1	0	4	1	4	2	1	4	0	1	26	2	1
78	207392C	8	1	0	1	1	1	1	1	1	0	1	6	2	1
Sno	**Hos.No**	**Age**	**Sex**	**Mstatus**	**Edu**	**Locat**	**Diag**	**Source**	**HLA**	**Bld grp**	**Drug aller**	**Bld trans**	**D_Age**	**D_Sex**	**Relat**
79	291985D	6	2	0	1	1	1	1	1	1	0	1	2	1	2
80	616661D	2	2	0	0	1	3	2	1	4	0	1	7	1	2
81	575339D	44	1	1	3	1	4	2	1	2	0	1	47	2	1
82	463024D	45	1	1	6	1	12	2	1	1	0	1	44	1	2
83	602097D	29	1	1	6	1	3	2	1	1	0	1	25	1	2
84	505773D	50	2	1	5	1	3	2	2	1	0	1	48	1	0
85	606551D	28	1	1	5	1	4	2	1	2	0	1	26	2	1
86	619327D	44	2	1	5	1	2	2	1	2	0	1	48	2	1
87	334171C	13	1	0	2	1	8	2	1	1	0	1	9	2	1
88	475365D	1	1	0	0	1	14	1	7	1	0	1	33	1	3
89	487826D	25	2	1	5	1	3	2	1	1	0	1	39	1	2
90	271409D	6	1	0	0	1	1	1	1	4	0	1	13	1	2
91	409351D	8	2	0	1	1	5	2	1	1	0	1	4	2	1
92	607417D	28	1	2	6	1	2	2	1	2	1	1	34	2	1
93	619264C	21	2	2	5	1	4	2	1	1	0	1	23	1	2
94	530345D	46	1	1	3	2	2	2	1	2	0	1	49	2	1
95	612836D	20	1	0	4	1	3	2	4	2	0	1	14	1	2
96	633221D	37	1	1	5	1	4	2	1	6	0	1	48	2	1
97	431214D	2	1	0	0	1	1	1	1	3	0	1	6	1	2
98	465204C	8	2	0	1	1	1	1	1	1	0	1	2	2	1
99	413636D	20	1	0	5	1	5	2	5	2	1	0	38	2	0
100	660012D	13	1	0	2	1	4	2	1	2	1	1	15	2	1
101	182812D	6	1	0	0	2	1	1	1	1	0	1	8	2	1
102	541802D	17	2	0	3	1	3	2	5	2	1	0	42	1	0

Sno	Bld grp	Preg	Cond	Px	Rx	E_ANC	E-Plts	Ac GVHD	Chr GVHD	Liver	Gut	FU1	GVHD	Gd	Bx Gd	FU2	GVHD
1	2	0	1	1	1	15	14	0	1	1	0	35	0				
2	4	0	2	1	0	15	36	0	0	0	0	15	0				
3	4	0	1	1	1	11	11	1	1	0	1	27	0				
4	1	0	3	1	3	18	0	1	0	1	1	11	0				
5	2	0	4	1	3	12	13	1	1	1	1	14	0			61	1
6	4	0	5	1	0	19	11	0	0	0	0	5	0				
7	5	0	6	1	1	15	14	1	0	1	1	7	0				
8	2	0	1	1	3	14	14	1	1	1	1	4	0			172	3
9	2	1	6	1	2	17	28	1	0	0	1	10	0				
10	2	0	1	1	0	13	0	0	0	0	0	7	0				
11	1	0	7	1	3	13	10	1	1	1	1	11	0				
12	4	0	8	1	0	17	49	0	0	0	0	7	0				
13	4	0	6	1	2	14	14	1	0	0	1	3	0				
14	2	0	2	1	0	19	30	0	0	0	0	12	0				
15	4	0	1	1	1	13	14	1	0	1	1	3	0				
16	4	0	6	1	1	17	25	1	0	0	1	6	0				
17	2	1	1	1	2	11	10	1	1	1	0	7	0				
18	6	0	1	1	1	12	12	1	1	0	1	13	0				
19	1	0	1	1	0	13	13	0	1	0	0	7	0				
20	1	0	2	1	0	18	41	0	0	0	0	5	0				
21	4	0	3	1	0	0	0	0	0	0	0	4	0				
22	7	-	3	1	0	18	18	0	0	0	0	26	0				
23	4	0	9	1	0	0	0	0	0	0	0	3	0				
24	2	0	2	1	1	17	43	1	0	0	1	12	0				
25	3	0	8	1	0	11	12	1	0	1	0	21	2	2	2		
26	1	0	7	2	0	0	0	0	0	0	0	3	0				
27	2	0	2	1	0	21	45	0	0	0	0	21	0				
28	1	0	10	1	0	11	8	0	1	0	0	13	0				
29	2	0	7	1	1	11	13	1	0	1	1	25	0			67	1
30	1	0	6	1	0	0	0	0	0	0	0	10	0				
31	1	0	10	1	3	32	10	1	0	1	1	18	2	2	3		
32	2	0	7	1	1	13	12	0	0	0	0	25	0				
33	1	0	1	1	1	13	13	1	0	1	0	18	0				
34	2	0	2	1	1	15	31	0	0	0	0	19	0				
35	1	0	2	1	0	19	47	0	0	0	0	29	0				
36	5	0	2	1	2	17	32	0	0	0	0	24	0				
37	2	0	2	1	0	17	40	0	0	0	0	22	0				
38	4	0	6	1	0	12	13	0	0	0	0	22	0				

39	6	0	1	1	1	17	25	0	1	0	1	35	0			283	3
Sno	**Bld grp**	**Preg**	**Cond**	**Px**	**Rx**	**E_ANC**	**E-Plts**	**Ac GVHD**	**Chr GVHD**	**Liver**	**Gut**	**FU1**	**GVHD**	**Gd**	**Bx Gd**	**FU2**	**GVHD**
40	1	0	2	1	0	0	0	0	0	0	0	22	0				
41	1	0	11	1	0	13	16	1	0	0	0	21	0			32	2
42	1	0	1	1	0	17	10	1	0	0	0	21	2	1	2		
43	2	0	2	1	2	19	45	0	0	0	0	17	0				
44	2	1	12	5	0	0	0	0	0	0	0	27	0				
45	4	0	2	1	0	15	36	1	0	0	0	20	2	2	1		
46	4	0	13	1	3	12	12	1	0	1	1	11	0				
47	2	0	14	1	0	20	57	1	1	1	0	22	0				
48	4	0	3	1	3	16	17	1	0	1	0	15	2	3	1		
49	1	0	7	1	3	16	47	0	0	0	0	28	0				
50	1	0	7	1	1	16	12	0	0	0	0	19	0				
51	1	1	2	1	0	0	0	0	0	0	0	10	0				
52	3	0	7	1	1	15	12	1	1	0	1	11	0				
53	4	0	6	1	3	14	13	1	0	0	1	59	0			70	2
54	1	0	3	1	4	24	29	1	0	1	1	11	0				
55	6	0	2	1	0	16	14	0	0	0	0	24	0				
56	4	0	6	1	1	28	0	1	0	1	1	21	2	3	1		
57	1	1	1	1	3	28	14	1	0	1	1	16	0				
58	2	1	8	3	3	17	10	1	0	1	0	29	0				
59	4	0	13	1	0	17	15	0	0	0	0	24	0				
60	4	-	5	1	3	13	17	1	0	1	1	21	0				
61	4	0	1	1	0	12	0	0	0	0	0	15	0				
62	1	0	1	1	1	9	15	0	0	0	0	30	0				
63	2	0	2	1	1	16	33	1	1	1	1	16	2	1	3		
64	2	0	13	1	0	16	15	0	0	0	0	24	0				
65	2	0	6	1	0	16	13	0	1	0	0	24	0			178	3
66	4	0	6	1	0	15	18	0	0	0	0	2	0				
67	1	0	7	1	3	15	13	1	0	1	1	11	2	1	2		
68	1	0	1	1	1	13	12	1	0	0	1	30	0				
69	2	1	8	1	1	9	0	0	0	0	0	24	0				
70	1	1	1	1	3	14	13	1	1	1	1	15	2	3	1	166	2
71	1	0	13	1	1	0	0	1	0	0	0	14	2	1			
72	1	0	13	1	1	14	21	0	0	0	0	7	0				
73	1	0	1	1	1	16	21	1	1	0	1	28	0			48	3
74	8	0	13	1	0	20	26	0	0	0	0	24	0				
75	1	0	13	1	0	18	38	1	0	0	0	3	0			49	3
76	2	0	8	1	0	20	35	0	0	0	0	25	0				

77	4	1	6	1	0	13	13	0	0	0	0	21	0				
78	1	0	2	1	0	20	0	0	0	0	0	24	0				
Sno	**Bld grp**	**Preg**	**Cond**	**Px**	**Rx**	**E_ANC**	**E-Plts**	**Ac GVHD**	**Chr GVHD**	**Liver**	**Gut**	**FU1**	**GVHD**	**Gd**	**Bx Gd**	**FU2**	**GVHD**
79	1	0	2	1	0	19	28	0	0	0	0	21	0				
80	2	0	15	2	1	13	12	1	0	0	1	8	0				
81	2	1	6	1	0	14	17	0	0	0	0	31	0				
82	1	0	1	1	3	17	24	1	0	1	1	28	0				
83	1	0	7	1	0	14	18	0	0	0	0	21	0				
84	1	0	7	1	1	15	31	1	0	0	1	24	0				
85	2	1	6	1	0	18	19	0	0	0	0	24	0				
86	4	1	7	1	1	14	18	1	0	1	0	18	0				
87	2	0	10	1	0	20	10	0	0	0	0	17	0				
88	1	0	2	2	0	25	0	1	0	1	1	21	0				
89	1	0	7	1	1	14	11	1	0	1	0	18	0				
90	6	0	13	1	3	18	19	1	0	1	0	21	0			35	2
91	3	0	1	1	0	16	61	0	0	0	0	26	0				
92	2	1	1	1	1	15	19	1	0	1	0	25	0			46	1
93	1	0	6	1	3	16	28	1	0	0	1	19	0				
94	2	1	16	4	3	12	19	1	0	1	1	19	0			34	3
95	2	0	7	1	3	13	17	1	0	0	0	9	0			32	3
96	1	1	6	1	1	12	20	1	0	0	1	26	0				
97	4	0	17	1	0	19	0	0	0	0	0	11	0				
98	1	0	13	1	0	57	36	0	0	0	0	19	0				
99	2	-	7	1	3	17	16	1	0	0	0	5	0			26	3
100	3	0	6	1	0	14	19	0	0	0	0	21	0				
101	1	0	18	1	0	17	22	0	0	0	0	14	0				
102	1	0	7	1	5	19	14	1	0	1	1	18	2	1	2	20	2

Sno	Gd	Bx Gd	FU3	GVHD	Gd	Bx Gd	FU4	GVHD	Gd	Bx Gd	Relap	Exp
1							497	3	1	2		
2							505	0				
3			170	1			515	0				
4							81	0				1
5		2	140	1		3	492	2	1			
6							91	0			1	1
7							462	0				
8	1	3					312	0				
9							87	0				1
10							13	0				1
11							322	3	1	2		
12							456	0				
13							100	0				1
14							451	0				
15							437	0				
16							436	0				
17							402	3	1	2		
18			377	2	1	2	419	2	1			
19							432	3	1	2		
20							397	0				
21							8	0				1
22							65	0				1
23							9	0				1
24							379	0				
25							336	0				1
26							8	0				1
27							370	0				
28							376	1				
29		2	291	1			387	0			1	
30							12	0				1
31			78	3	1	3	217	1				
32							373	0			1	
33							157	0				1
34							45	0				1
35							352	0				
36							348	0				
37							346	0				
38							295	0				

39	1		322	1			341	0				
Sno	**Gd**	**Bx Gd**	**FU3**	**GVHD**	**Gd**	**Bx Gd**	**FU4**	**GVHD**	**Gd**	**Bx Gd**	**Relap**	**Exp**
40							33	0				1
41	1	3					136	0				1
42							287	0				
43							80	0			1	1
44							46	0				1
45							285	0				
46							296	0				
47							285	2	2	Chronic Lichenoid		
48			34	2	3	3	241	0				
49							242	0				
50							25	0				
51							15	0				1
52			231	3	1		289	3	1			
53	3						269	0				
54							58	0				1
55							271	0				
56							257	0				
57							107	3	1	2		1
58							47	0				1
59							66	0				
60							43	0				1
61							18	0				1
62							249	0				
63							210	0				
64							217	0				
65	1	2	199	3	1		236	3	1			
66							233	0				
67							233	0				
68							228	0				
69							53	0				1
70	1		194	2	3	3	220	2	3			
71							46	0				1
72							161	0				
73	1	3	130	2	3	1	191	0				
74							138	0				
75	1	2	49	3	1	2	154	0				
76							191	0				

77							182	0				
78							33	0				1
Sno	**Gd**	**Bx Gd**	**FU3**	**GVHD**	**Gd**	**Bx Gd**	**FU4**	**GVHD**	**Gd**	**Bx Gd**	**Relap**	**Exp**
79							159	0				
80							33	0				
81							169	0				
82							166	0				
83							170	0			1	
84							162	0				
85							122	0				
86			60	3	1	2	137	1				
87							141	0				
88							33	0				1
89			95	3	1	2	142	3	1			
90	3	1	77	3	3		105	3	3			
91							136	0			1	
92	1	2					135	3	1			
93							122	0				
94	3	3					78	0				1
95	2	2	46	3	1		121	0				
96							113	0				
97							92	0				
98							89	0				
99	3	3	46	3	3		107	2	3	2		
100							96	0				
101							82	0				
102	4						93	0				